RADIONUCLIDES METHODS IN VITRO
clearance measurement methods
Question 1

What is the best index for renal function? *(one choice only)*

A – renal size

B – urine flow

C – renal blood/plasma flow

D – glomerular filtration rate

E – filtration fraction
Question 2

What is the best technique to assess glomerular filtration rate? (one choice only)

A – serum creatinine assay
B – MDRD formula
C – blood urea nitrogen
D – 99mTc-DMSA absolute uptake
E – plasma clearance of $^{51}$Cr-EDTA
Question 3

Should we normalise GFR to body size?
(one choice only)

A – no, never

B – yes, always, to body surface area

C – yes, most of the time, to body surface area

D – yes, always, to body weight

E – yes, most of the time, to body weight
Question 4

What could best characterise urine clearance measurements, as compared to plasma clearance measurements? *(one choice only)*

A – they are more precise and more accurate
B – they are less precise but more accurate
C – they are more precise but less accurate
D – they are less precise and less accurate
E – they are more precise and as accurate
Question 5

In which circumstance(s) should you perform a urinary clearance measurement instead of a plasma clearance measurement? *(potentially several answers)*

A – in children

B – in patients with œdema

C – in patients with hyperfiltration

D – in patients with very low renal function

E – in patients with asymmetrical renal function
Question 6

What is the general formula for clearance?
P: plasma concentration – U: urinary concentration
(one choice only)

A – P × U / V
B – U × V / P
C – U × V / BW
D – (C × O / V ) × (P / U)
E – P × V × U
Question 7

How is the plasma clearance determined?
(one choice only)

A – the urinary concentration divided by the plasma concentration
B – the area under the plasma time-concentration curve divided by the injected activity
C – the area under the plasma time-concentration curve divided by the plasma concentration at time 0
D – the injected activity divided by the area under the plasma time-concentration curve
E – the injected activity divided by the area under the plasma time-concentration curve
Question 8

When using two plasma samples for plasma clearance technique:
(*potentially several answers*)

A – two exponentials must be determined

B – one exponential only can be determined

C – a correction formula must be used, the recommended one being published by Brochner and Mortensen

D – a correction formula must be used, the recommended one being published by Christensen and Groth

E – there is no need for correction
Clinical indications for renal clearance measurements

The concept of renal clearance

- renal function
- clearance
- glomerular

Methods of clearance measurements

- tracers
- types of clearances
- urinary clearance
- continuous infusion plasma clearance
- single shot plasma clearance
- normalisation for body size

Practical issues in measurement

Choice of Method

Interpretation

- normal values
- body size scaling
- case of children
CLINICAL INDICATIONS

- **global function**: clearance
- **relative function**: renal scan
- **individual function**: clearance + renal scan

[Link to Strauss's website](http://www.bio.psu.edu/people/faculty/strauss)
CLINICAL INDICATIONS

• can a patient withstand nephrectomy (either for himself or as a kidney donor)
• can a patient withstand nephrotoxic drugs (anti-tumoural chemotherapy)?
• adapt drug dosage to renal function
• detect mild renal insufficiency
• renal function follow-up
• prepare dialysis
• can a patient stop dialysis?
• how does a patient behaves under a drug (i.e. ACE inhibitors)
• concept of glomerular functional reserve
• determine hyperfiltration in diabetics
concept of glomerular functional reserve

After infusion of:
• either Dopamin 2 µg/kg/min
• or some amino-acids (Hyperamin 50 mL/hr)
• or both

glomerular filtration rate increases by about 15%
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  clearance
  glomerular

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  case of children
CONCEPT: function

- keep homeostasis (water, ions, )
- excretion of toxic substances
- metabolism (1-hydroxylation of vitamin D)
- hormonal: erythropoietin, renin

best parameter to assess renal function =

**Glomerular Filtration Rate (GFR)**

Levey AS.

*Use of glomerular filtration rate measurements to assess the progression of renal disease*


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CONCEPT: clearance

renal (arterial) plasma flow

$F$

$P_A$

plasma (arterial) concentration of a substance

renal (venous) plasma flow

$F$

$P_V$

plasma (venous) concentration of the substance

extraction:

$$E = \frac{P_A - P_V}{P_A} = 1 - \frac{P_V}{P_A}$$

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CONCEPT: clearance

renal (arterial) plasma flow

$F$

renal (venous) plasma flow

$F$

$200 \text{ mmol/L}$

$50 \text{ mmol/L}$

extraction:

$$E = \frac{200 - 50}{200} = 1 - \frac{50}{200} = 75\%$$
CONCEPT: clearance

**substance arterial flow**

\[ P_A \times F \]

**substance venous flow**

\[ P_V \times F \]

**substance urinary flow**

\[ U \]

**urine flow**

\[ V \]

**urinary concentration**

\[ UV \]
CONCEPT: clearance

substance arterial flow

\[ P_A \times F \]

substance venous flow

\[ P_V \times F \]

substance urinary flow

\[ UV \]

\[ P_A \times F - P_V \times F = UV \]
CONCEPT: clearance

\[ P_A \times F - P_V \times F = UV \]
\[ E = \frac{P_A - P_V}{P_A} \]
\[ (P_A - P_V) \times F = UV \]
\[ P_A - P_V = E \times P_A \]
\[ E \times P_A \times F = UV \]
\[ E \times F \times P = UV \]
CONCEPT: clearance

100 mmol/min.
substance arterial flow

500 mL/min

200 mmol/L
= 0.2 mmol/mL

1.5 mL/min
= 2 L/day

25 mmol/min.
substance venous flow

50 mmol/L
= 0.05 mmol/mL

75 mmol/min

50 mmol/mL

\[ E = 75\% \]

\[ P_A \times F \]

\[ P_V \times F \]

\[ E \times F \times P = UV \]

( imaginary substance )
CONCEPT: clearance

\[ C = \frac{U \times V}{P} \]

- **Clearance**: mL/min
- **U**: mmol/L
- **V**: mL/min
- **P**: mmol/L
CONCEPT: clearance

\[ C = \frac{U \times V}{P} \]

\[ E \times F \times P = UV \]

\[ C = \frac{E \times F \times P}{P} \]

\[ C = E \times F \]

clearance is the kidney ability to extract the substance multiplied by the kidney input i.e. plasma flow. The product corresponds to the kidney function.
CONCEPT: clearance

\[ C = \frac{U \times V}{P} \]

\[ C = E \times F \]

50 mmol/mL \times 1.5 \text{ mL/min} = 75 \text{ mmol/min}

375 \text{ mL/min}

200 mmol/L = 0.2 mmol/mL

500 \text{ mL/min}

75%
CONCEPT: clearance

urine is 250 times more concentrated than plasma

1 mL urine ‘cleans’ 250 mL plasma from substance

\[ C = \frac{U}{P} \times V \]

50 mmol/mL

375 mL/min

1.5 mL/min

0.2 mmol/mL

1.5 mL/min urine ‘cleans’ 250 x 1.5 = 375 mL/min plasma

*clearance is the imaginary plasma flow cleared from substance*

i.e. *kidney ability to ‘clean’ plasma from substance*
CONCEPT: clearance

Clearance:

\[ C = \frac{U \times V}{P} = E \times F \]

**definition:**
imaginary plasma flow (volume per unit time) completely cleared from substance

**for a given organ:**
ability to take the substance including the input function (perfusion)

= plasma flow × extraction
CONCEPT: clearance

Clearance reflects renal function as if the role of the kidney were to remove a substance from the blood (which is a shortcut)
Clinical indications for renal clearance measurements

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CONCEPT: glomerular

A glomerular tracer
is a small molecule, freely filtered, not linked to proteins
not secreted nor reabsorbed

its concentration in the plasma is $P$

its concentration in the glomerular filtrate (primary urine) is also $P$

its flow in the glomerular filtrate is $P \times \text{GFR}$

as it is not secreted nor reabsorbed,
its flow in the final urine is also $UV = P \times \text{GFR}$

its clearance is therefore $C = \frac{P \times \text{GFR}}{P} = \text{GFR}$
CONCEPT: glomerular

A glomerular tracer is a small molecule, freely filtered, not linked to proteins not secreted nor reabsorbed.

Its clearance is the glomerular filtration rate.
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« Gold Standard »: Inulin (cold, exogenous)
METHODS: tracers (inulin)

Exogenous cold tracer (former gold standard) = inulin

fructose polymer (polyfructosan)
extracted from Jerusalem artichoke
considered as a gold standard
though, also cleared by other organs
assays are not straightforward
no more widely available for in vivo human use
Routine: Creatinine (cold, endogenous)
METHODS: tracers (creatinine)

Endogenous tracer used in clinical routine: creatinine

- Endogeneous: no injection is needed
- Produced by protein catabolism
- Production is not constant (muscles/food intake)
- Not only filtered but secreted
- Assays are not straightforward (many interferences)
- Assay results strongly depend on technique

$$\begin{align*}
\text{amino acids} \\
\text{creatine} \\
\text{creatinine}
\end{align*}$$
METHODS: tracers (creatinine)

Endogenous tracer used in clinical routine: creatinine

How to interpret creatinine assays?

- urinary creatinine clearance
- plasma creatinine only
- plasma creatinine with formulae
  Cockroft and Gault, MDRD, Schwartz, Counahan-Barratt…
Using only the MDRD formula is still debated considered to be reliable within a ± 30% confidence interval! unsuitable to children, elder people, obese, very thin, patients with advanced renal of hepatic insufficiency…

Radiopharmaceuticals: glomerular
METHODS: tracers (radiopharmaceuticals, glomerular)

GLOMERULAR TRACERS

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Labelling</th>
<th>MW</th>
<th>Filtration fraction</th>
<th>Secretion</th>
<th>Extraction coefficient</th>
<th>Protein binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPA</td>
<td>diethylene pentacetic acid</td>
<td>99mTc, 169Yb, 113mIn, 140La</td>
<td>393 D</td>
<td>15-20%</td>
<td>–</td>
<td>15-20%</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene-diamine-tetracetic acid</td>
<td>51Cr</td>
<td>292 D</td>
<td>15-20%</td>
<td>–</td>
<td>15-20%</td>
</tr>
<tr>
<td></td>
<td>iothalamate</td>
<td>125I, 131I</td>
<td>614 D</td>
<td>15-20%</td>
<td>10-20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>diatrizoate</td>
<td>125I</td>
<td>636 D</td>
<td>15-20%</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

ideal: filtered, non-secreted, non reabsorbed, no protein binding

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METHODS: tracers (radiopharmaceuticals, glomerular)

EDTA

DTPA (diethylenetriaminepentaacetic acid)
METHODS: tracers

$^{51}\text{Cr}$-EDTA (Ethylene-Diamine-TetrAcetate)

$^{51}\text{Cr}$: $T_{1/2} = 27.7$ j

CE + $\gamma$ 320 keV (10%)

no imaging, only clearance

already labeled in 1 mCi vials

excellent *in vitro* and *in vivo* stability

present gold standard

(small extra-renal clearance $\sim 4$ mL/min.)

$^{99m}\text{Tc}$-DTPA (Diethylene-Tetramine-PentAcetate)

imaging and/or clearances
cold kit

*good in vitro* and *in vivo* stability

protein binding should be checked for (depends on brand used)

labeling yield (free Tc and reduced-hydrolysed Tc)
Among Glomerular tracers:

- **EDTA-\(^{51}\)Cr** is a tracer of choice (unavailable in the USA)
- **DTPA-\(^{99m}\)Tc** is good provided protein binding is checked (strong variations among preparations)
- Iothalamate is only a second choice (USA)
  - it is also secreted

*Rehling - Scand J Clin Lab Invest 1988; 48: 603*
*Rehling - Nucl Med Commun 1997; 18: 324*
*Brøchner – Clin Physiol 1985; 5: 1*
**METHODS: tracers (radiopharmaceuticals, glomerular)**

Other Glomerular Tracers…

<table>
<thead>
<tr>
<th>Iodinated Contrast Media</th>
<th>Iohexol (OK if HPLC assays)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cold Iothalamate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radiopharmaceuticals</th>
<th>Labelled Iothalamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diatrizoate</td>
</tr>
</tbody>
</table>
Clinical indications for renal clearance measurements

The concept of renal clearance
- renal function
- clearance
- glomerular

Methods of clearance measurements
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- urinary clearance
- continuous infusion plasma clearance
- single shot plasma clearance
- normalisation for body size

Practical issues in measurement

Choice of Method

Interpretation
- normal values
- body size scaling
- case of children
METHODS: types of clearances

- plasma clearance
- external counting
- urinary clearance (renal clearance)
METHODS: types of clearances

- **bolus** ("single shot")
- **continuous infusion**
METHODS: types of clearances

- **Single shot**
  - Plasma clearance

- **Continuous infusion**
  - Plasma clearance
  - External counting
  - Urinary clearance (renal clearance)
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Choice of Method

Interpretation
normal values
body size scaling
case of children
METHODS: urinary

- single shot
- continuous infusion

OR

plasma clearance
external counting
urinary clearance (renal clearance)
METHODS: urinary

\[ GFR = \frac{U \times V}{P} \]

- urinary concentration
- urinary flow
- plasma concentration
METHODS: urinary

\[ C = \frac{U \times V}{P} \]

plasma concentration \( P \)

time \( t \)

micturition

\((UV)_1\) \(\rightarrow\) \(P_1\)
\((UV)_2\) \(\rightarrow\) \(P_2\)
\((UV)_3\) \(\rightarrow\) \(P_3\)
\((UV)_4\) \(\rightarrow\) \(P_4\)
METHODS: urinary

In fact, any scheme of injection suits (IV, infusion, even SC) hydrate well (urine flow should exceed 3 mL/min)

plasma sampling

urine collection

individual GFR determination for each collection period

value averaging (discard when flow < 1 mL/min)
**METHODS: urinary**

**exemple:**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>9h00</td>
<td>injection</td>
<td>1200 cpm/mL</td>
<td></td>
</tr>
<tr>
<td>10h00</td>
<td>micturition (thrown away)</td>
<td>35 000 cpm/mL</td>
<td>52 mL</td>
</tr>
<tr>
<td>10h30</td>
<td>plasma sampling</td>
<td>800 cpm/mL</td>
<td></td>
</tr>
<tr>
<td>11h00</td>
<td>micturition</td>
<td>20 000 cpm/mL</td>
<td>240 mL</td>
</tr>
<tr>
<td>11h30</td>
<td>plasma sampling</td>
<td>600 cpm/mL</td>
<td></td>
</tr>
<tr>
<td>12h00</td>
<td>micturition</td>
<td>15 000 cpm/mL</td>
<td>180 mL</td>
</tr>
</tbody>
</table>
METHODS: urinary

\[ P_1 = 1200 \text{ cpm/mL} \]
\[ U_1 = 35000 \text{ cpm/mL} \quad V_1 = 52 \text{ mL/60 min} \quad < 1 \text{ mL/min} \]
\[ P_2 = 800 \text{ cpm/mL} \]
\[ U_2 = 20000 \text{ cpm/mL} \quad V_2 = 240 \text{ mL/60 min} \]
\[ P_3 = 600 \text{ cpm/mL} \]
\[ U_3 = 15000 \text{ cpm/mL} \quad V_3 = 180 \text{ mL/60 min} \]

\[ Cl = \frac{20000 \text{ cpm/mL} \times 4 \text{ mL/min}}{800 \text{ mL/min}} \]
\[ Cl = \frac{15000 \text{ cpm/mL} \times 3 \text{ mL/min}}{600 \text{ mL/min}} \]

averaging 100 and 75 = 87 \text{ mL/min}
METHODS: urinary

Urinary clearance is:

**accurate**: no bias (no extrarenal clearance)

**imprecise**: scatter (urine collection is troublesome)

*(experimental) gold standard = urethral catheterisation + bladder rinsing + air exsufflation*

accurate
not precise
(like urinary cl.)

precise
not accurate
(like plasma cl.)
Clinical indications for renal clearance measurements

The concept of renal clearance
   renal function
   clearance
   glomerular

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Practical issues in measurement

Choice of Method

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METHODS: continuous infusion plasma clearance

Instead of measuring the urine output, we measure the infusion input.
METHODS: continuous infusion plasma clearance

\[ GFR = \frac{\text{flow}}{\text{plateau}} \]

\[ C = \frac{R \times V}{PP} \]

at the plateau, input = output
METHODS: continuous infusion plasma clearance

\[ C = \frac{R}{P} \]

\[ P = 500 \text{ cpm} / \text{mL} \]

\[ R = 50,000 \text{ cpm} / \text{min} \]

\[ GFR = \frac{50,000 \text{ cpm} / \text{min}}{500 \text{ cpm} / \text{mL}} = 100 \text{ mL/min} \]
METHODS: continuous infusion plasma clearance

In practice:

• roughly estimate GFR from creatinine
• inject a loading dose (22 kBq/kg BW)
• then infuse with constant flow (7 kBq/[ml/min GFR])
• plasma sampling between 1½ et 4 h after the infusion start
• no need for accurate timing
• urine collection is required if GFR<15 mL/min or ascites or oedema
• the infusion solution is calibrated by “infusing” tubes
METHODS: continuous infusion plasma clearance

- nearly no possible error (very robust)
- dynamic (baseline+test condition are feasible)
  (glomerular reserve/ACE inhibitors…)
- precise
- can be used with a urinary clearance

- requires a several hour infusion
- impurities may accumulate in the plasma
- cumbersome ?

- no precise timing needed
- standard calibration is very easy and robust
- trained nurses find it no more time-consuming than single-shot clearance
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Single injection plasma clearance

general principle
full sampling (two-exponential)
slope-intercept (single-exponential)
single point
slope-only
METHODS: bolus injection plasma clearance

\[ C = \frac{UV}{P} = \frac{U(t) \times V(t)}{P(t)} \]

\[ C \times P(t) = U(t) \times V(t) \]

tracer flow in urine

\[ C \times \int_0^\infty P(t) \, dt = \int_0^\infty U(t) \times V(t) \, dt = q \]

\[ C \times \int_0^\infty P(t) \, dt = q \]

\[ C = \frac{q}{\int_0^\infty P(t) \, dt} = \frac{\text{injected activity}}{\text{area under the plasma time-activity curve}} \]
METHODS: bolus injection plasma clearance

\[ Cl = \frac{q}{\int_{0}^{\infty} P(t) \, dt} \]

Q

plasma activity

time
METHODS: bolus injection plasma clearance

So, to measure plasma clearance, we need:

- to know how much tracer has been injected
  (seems easy… but tricky)

- to know the integral of plasma-time activity curve
  continuous infinite function so need:
  ➢ plasma sampling
  ➢ a mathematical model for the curve
Single injection plasma clearance

general principle
full sampling (two-exponential)
slope-intercept (single-exponential)
single point
slope-only
METHODS: bolus injection plasma clearance

Theory predicts that:

\[ P(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]

fast component \quad slow component
METHODS: bolus injection plasma clearance

\[ P(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]

- **fast component**
- **slow component**

Exact technique:
- take \( \gg 4 \) samples
- fit model curve (2 exp.) to experimental data
- determine the four parameters: \( A, B, \alpha, \beta \)
- calculate the integral:
  \[ \int_0^\infty P = \frac{A}{\alpha} + \frac{B}{\beta} \]

- infer clearance
  \[ C = \frac{q}{\infty} = \frac{q}{\int_0^\infty P} = \frac{q}{\frac{A}{\alpha} + \frac{B}{\beta}} \]

*Sapirstein – Am J Physiol 1955; 181: 330*
METHODS: bolus injection plasma clearance

\[ Cl = \frac{q}{\int_0^{+\infty} P(t) \, dt} \]

- fast removal
- small area
- high function

- slow removal
- large area
- low function
METHODS: bolus injection plasma clearance

Express injected activity in the same way as the activity per unit of volume, usually detected activity:
i.e. counts per minute (cpm) but might be Bq, g, mol…

\[
Cl = \frac{q}{\int_{0}^{+\infty} P(t) \, dt} = \frac{10^7 \text{ cpm}}{(10^5 \text{ cpm} \cdot \text{mL}^{-1}) \cdot \text{min}} = 100 \text{ mL} \cdot \text{min}^{-1}
\]
METHODS: bolus injection plasma clearance

For this, use a ‘standard’: known volume of water, ‘injected’ like the patient counted like the patient’s plasma.
METHODS: bolus injection plasma clearance

Calibration needs comparison between injected activities (patient/standard)

\[ q_{\text{Cl}} \int_0^\infty P(t) \, dt = \int_0^\infty \text{counting}_{\text{patient}} - \text{counting}_{\text{standard}} \]

E.g. 10 000 000 cpm

E.g. 3 000 cpm/mL at time t
METHODS: bolus injection plasma clearance

Calibration needs comparison between injected activities (patient/standard)

\[ \text{ratio by comparing:} \]
- masses
- or volumes
- or activities

\[ Cl = \frac{q}{\int_0^{+\infty} P(t) dt} \]
METHODS: bolus injection plasma clearance

In practice, for the two-exponential technique:

- sample from 5-10 min. post injection
- up to 3-24 h according to expected renal function
- 8 samples is good
- cumbersome → alleviate burden is desirable

… simplified techniques!
Single injection plasma clearance

general principle
full sampling (two-exponential)
slope-intercept (single-exponential)
single point
slope-only
METHODS: bolus injection plasma clearance

Mono-exponential technique (‘slope-intercept’)

\[ P(t) = \frac{A}{1} e^{-\alpha t} + \frac{B}{1} e^{-\beta t} \]

- **fast component**
- **slow component**

late sampling only
use only one exponential
compensate for neglecting the first exponential
METHODS: bolus injection plasma clearance

\[ P(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]

\[ P(t) = B \cdot e^{-\beta t} \]

\[ Cl = \frac{q}{\int_{0}^{+\infty} P(t) \, dt} \]

The measured \( P(t) \) is lower than the true \( P(t) \)
Denominator is underestimated
Clearance is overestimated
Calculated clearance should be reduced
The higher the renal function, the higher the error
METHODS: bolus injection plasma clearance

Calculated clearance should be corrected (reduced):

- multiply by a number < 1 (Chantler):
  0.93 for adults / 0.87 for children

- use a 2\textsuperscript{nd} degree formula: ax\textsuperscript{2}+bx (Brøchner-Mortensen)

- use a more physiological formula (recommended)
  Fleming
  improved by Jødal and Brøchner-Mortensen

\[
\frac{C_{L_{\text{corrected}}}}{C_{L_{\text{uncorrected}}}} = \frac{1}{1 + f \times C_{L_{\text{uncorrected}}}}
\]

\[
f = 0.0032 \text{ m}^{0.65} \times BSA^{-1.3}
\]

\textit{here the clearance values are not normalised to BSA}


\textit{Fleming: same assuming BSA is 1.33 m\textsuperscript{2}}

(calculations after normalisation to BSA)
METHODS: bolus injection plasma clearance

In practice, for slope-intercept technique (mono-exponential):

• sample 2-4 samples starting no earlier than 60-90 min
• fit the plasma activity decay to a single exponential
• calculate the uncorrected clearance value

\[
C = \frac{q}{B} = \frac{\alpha/\beta}{B} + \frac{B}{\phi/\beta}
\]

• compensate for single exponential
  (preferably with Jødal formula)
METHODS: bolus injection plasma clearance

With slope-intercept technique, at least two-samples are required. Can it be simplified further? In principle, no! Two unknowns: ECV and GFR so two samples are needed.

However, if one makes an assumption about ECV, GFR can then be inferred with only one sample: this is the principle of single-sample techniques.
Single injection plasma clearance

general principle
full sampling (two-exponential)
slope-intercept (single-exponential)
single point
slope-only
METHODS: bolus injection plasma clearance

Sampling at time $t$.
Plasma concentration of tracer is $P(t)$.
Nearly all these single sample-technique use the following intermediate parameter: apparent dilution volume:

$$V_D(t) = \frac{q}{P(t)}$$

The higher the plasma concentration, the smaller the volume
the smaller the clearance.
**METHODS: bolus injection plasma clearance**

Many formulæ were published (most were empirical):

<table>
<thead>
<tr>
<th>Category</th>
<th>Formula</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Fisher and Veall</td>
<td>1975</td>
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<tr>
<td></td>
<td>Morgan</td>
<td>1977</td>
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<tr>
<td></td>
<td>Constable</td>
<td>1979</td>
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<tr>
<td></td>
<td>Jacobsson</td>
<td>1983</td>
</tr>
<tr>
<td></td>
<td>Russel</td>
<td>1985</td>
</tr>
<tr>
<td></td>
<td>Christensen and Groth</td>
<td>1986</td>
</tr>
<tr>
<td></td>
<td>d° simplified by Watson</td>
<td>1992</td>
</tr>
<tr>
<td>Children</td>
<td>Groth</td>
<td>1984</td>
</tr>
<tr>
<td></td>
<td>Tauxe</td>
<td>1987</td>
</tr>
<tr>
<td></td>
<td>Ham and Piepsz</td>
<td>1991</td>
</tr>
<tr>
<td>General</td>
<td>Waller</td>
<td>1987</td>
</tr>
<tr>
<td></td>
<td>Russell</td>
<td>1985</td>
</tr>
<tr>
<td></td>
<td>Fleming</td>
<td>2005</td>
</tr>
</tbody>
</table>
METHODS: bolus injection plasma clearance

If we assume a single-exponential decay:

\[
P(t) = B \cdot e^{-\beta t} \quad P(0) = B
\]

\[
ECV = V_D(0) = \frac{q}{P(0)} = \frac{q}{B} \quad B = \frac{q}{ECV}
\]

\[
V_D(t) = \frac{q}{P(t)} = \frac{q}{B \cdot e^{-\beta t}} = \frac{q}{ECV} \cdot e^{-\beta t} = \frac{ECV}{e^{-\beta t}}
\]

\[
e^{-\beta t} = \frac{ECV}{V_D(t)} \quad -\beta t = \ln \frac{ECV}{V_D(t)} \quad \beta = \frac{-\ln \frac{ECV}{V_D(t)}}{t}
\]
If we assume a single-exponential decay:

\[ ECV(t) = \frac{q}{B} e^{-\beta t} \quad P(0) = B \]

\[ ECV = V_D(0) = \frac{q}{P(0)} = \frac{q}{B} \quad B = \frac{q}{ECV} \]

\[ V_D(t) = \frac{q}{P(t)} = \frac{q}{B \cdot e^{-\beta t}} = \frac{GFR}{ECV} \frac{q}{B} e^{-\beta t} = \frac{ECV}{e^{-\beta t}} \]

\[ e^{-\beta t} = \frac{ECV}{GFR} \frac{q}{B} = e^{-\beta t} \frac{ECV}{GFR} \frac{q}{B} = -\beta t \frac{ECV}{GFR} \frac{q}{B} = + \ln \frac{V_D(t)}{ECV} - \beta t \frac{ECV}{GFR} \frac{q}{B} \]

\[ V_D(t) = \ln \frac{ECV}{t} \]

Jacobsson 1983 Clin Physiol 3 297-305
METHODS: bolus injection plasma clearance

Assuming ECV, Christensen and Groth devised a technique, which was simplified by Watson to get the same result. The technique reduces in solving a 2\textsuperscript{nd} degree equation*:

\[ ax^2 + bx + c = 0 \]

\[ GFR = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \]

\[
\begin{align*}
  a &= 1.710^{-6} \times t^2 - 0.0012 \times t \\
  b &= -7.75 \times 10^{-4} \times t^2 + 1.31 \times t \\
  c &= \ln\left(\frac{ECV}{V_D(t)}\right) \times ECV
\end{align*}
\]

\[ ECV \approx 8116.6 \text{ mL/m}^2 \times BSA - 28.2 \text{ mL} \]

- recommended by the international consensus (Blaufox, Santa Fe, 1996)

Christensen & Groth – Clin Physiol 1986; 6: 579
METHODS: bolus injection plasma clearance

For children:


\[ GFR = 2.602 \times V_D(120 \text{ min}) - 0.273 \]

showed good precision even if empirical formula.

or

Fleming et al 2005 Nucl Med Comm 26 743-748

\[ GFR = \frac{a + b \times \ln\left(V_D(t) \times \frac{1.73 \text{ m}^2}{BSA}\right)}{t} \]

\[ a = -11297 \quad -4883 \times BSA \quad -41.9 \times t \]

\[ b = 5862 \quad +1282 \times BSA \quad +15.5 \times t \]
METHODS: bolus injection plasma clearance

Can we go further and determine GFR without even any plasma sample?

…unfortunately no!
Single injection plasma clearance

- general principle
- full sampling (two-exponential)
- slope-intercept (single-exponential)
- single point
- slope-only
METHODS: bolus injection plasma clearance

A few techniques were proposed using the slope only:
  Galli
  Peters *Peters - Nephrol Dial Transplant 1992; 7: 205*

These techniques have not been validated.
METHODS: bolus injection plasma clearance

Gamma-camera techniques (e.g. Gates, absolute DMSA,…):

good for **relative** measurements

**not recommended** for **absolute** measurements


+ many experimental studies

no more precise than creatinine-based techniques
Clinical indications for renal clearance measurements

The concept of renal clearance
renal function
clearance
glomerular

Methods of clearance measurements
tracers
types of clearances
urinary clearance
continuous infusion plasma clearance
single shot plasma clearance
normalisation for body size

Practical issues in measurement

Choice of Method

Interpretation
normal values
body size scaling
case of children
CHOICE OF METHOD

- EDTA or DTPA?
- plasma or urinary
- single-shot or infusion
- how many samples?
- when sampling?

- plasma clearance
- external counting
- urinary clearance (renal clearance)
CHOICE OF METHOD

First of all, collect information:
- indication
- age
- body mass
- height
- gender
- plasma creatinine
- presence of oedema/ascitis/3rd compartment
- (urea)
- (Black?)
- (albumin)

and estimate GFR from creatinine
(MDRD in adults / Schwartz in children)

ensure steady-state conditions
CHOICE OF METHOD

EDTA or DTPA?

both are good
DTPA can also be used for scanning
EDTA is a very stable tracer
DTPA should be checked for protein binding
CHOICE OF METHOD

plasma or urinary?

Plasma is more precise
But urinary is more accurate and mandatory if any of:
• expected GFR < 25 mL/min/1.73 m²
• ascites
• œdema
• third compartment
CHOICE OF METHOD

single-shot or infusion?

Single-shot is the most widely used

But continuous infusion is needed for:
  • assessment of glomerular functional reserve
  • assessment of GFR variations over various conditions
    (ACE inhibitors…)

Continuous infusion is very robust (kidney donors)
CHOICE OF METHOD

When single-shot is chosen

1 sample is precise but it is not robust
   (international consensus: Christensen & Groth / Watson)

2+ samples is more robust
   (British consensus: single-exponential + Brochner-Mortensen)
   2 samples make it possible to check VEC
   3 samples show if 3 points are not aligned
   4+ samples make it possible to exclude an aberrant point
   adding samples does not increase precision much
CHOICE OF METHOD

When to sample?

For single exponential: from 90 minutes post injection

For last sample
If expected GFR

- > 90 mL/min/1.73 m² up to 3 hr
- > 60 mL/min/1.73 m² up to 4 hr
- > 40 mL/min/1.73 m² up to 5 hr
- < 40 mL/min/1.73 m² up to 24 hr
METHODS: bolus injection plasma clearance

sample early (<4 h) if function expected normal

sample late (> 5h) if function expected low
If I want to start, what technique should I use in most cases?

- plasma clearance
- $^{51}\text{Cr-EDTA}$
- 3 samples
- single-exponential model (slope intercept)
- Jødal and Brøchner-Mortensen correction formula
Clinical indications for renal clearance measurements

The concept of renal clearance
  renal function
  clearance
  glomerular

Methods of clearance measurements
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Practical issues in measurement

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  normal values
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  case of children
normal values are difficult to establish:
• who is normal?
• is a normal truly normal?
• why carrying out a clearance measurement if a subject is normal?
• which technique was used (tracer/type of clearance/technique details)
• is GFR is the same in different populations?
### INTERPRETATION: normal values

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Age range (yrs)</th>
<th>No.</th>
<th>Substance</th>
<th>Mean GFR</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucht [8]</td>
<td>1951</td>
<td>18–44</td>
<td>27</td>
<td>Inulin</td>
<td>120</td>
<td>SD=18</td>
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<td>Smith [7]</td>
<td>1953</td>
<td>16–49</td>
<td>34</td>
<td>Inulin</td>
<td>124</td>
<td>SD=26</td>
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<tr>
<td>Brun et al. [9]</td>
<td>1953</td>
<td>20–25</td>
<td>10</td>
<td>Inulin</td>
<td>125</td>
<td>SEM=4</td>
</tr>
<tr>
<td>Findley [9]</td>
<td>1953</td>
<td>40</td>
<td>17</td>
<td>Inulin</td>
<td>117</td>
<td>/</td>
</tr>
<tr>
<td>Foà [9]</td>
<td>1953</td>
<td>22–56</td>
<td>7</td>
<td>Inulin</td>
<td>117</td>
<td>/</td>
</tr>
<tr>
<td>Berger [9]</td>
<td>1953</td>
<td>26</td>
<td>17</td>
<td>Inulin</td>
<td>126</td>
<td>SD=17</td>
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<tr>
<td>Merrill [9]</td>
<td>1953</td>
<td>32</td>
<td>129</td>
<td>Inulin</td>
<td>129</td>
<td>SD=40</td>
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<tr>
<td>Berglund et al. [11]</td>
<td>1976</td>
<td>50</td>
<td>78</td>
<td>EDTA</td>
<td>100</td>
<td>SD=12</td>
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<tr>
<td>Granerus and Aurell [14]</td>
<td>1981</td>
<td>26–33</td>
<td>33</td>
<td>EDTA</td>
<td>105</td>
<td>SD=26</td>
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<tr>
<td>Orlando et al. [17]</td>
<td>1998</td>
<td>54</td>
<td>16</td>
<td>Inulin</td>
<td>116</td>
<td>SD=10</td>
</tr>
<tr>
<td>Price et al. [19]</td>
<td>2001</td>
<td>38 ±1.8</td>
<td>82</td>
<td>Inulin</td>
<td>118</td>
<td>SEM=6</td>
</tr>
<tr>
<td>Vervoort et al. [20]</td>
<td>2002</td>
<td>28±6.1</td>
<td>46</td>
<td>Inulin</td>
<td>107</td>
<td>SD=11</td>
</tr>
<tr>
<td>Gurmandeep et al. [22]</td>
<td>2005</td>
<td>19–40</td>
<td>187</td>
<td>EDTA</td>
<td>103</td>
<td>SD=16</td>
</tr>
</tbody>
</table>

*SD* standard deviation, *SEM* standard error of the mean
INTERPRETATION : normal values

Various normal ranges, dependant:
  • on technique
  • on tracer
  • on population

Gender difference is debated:

men : $127 \pm 23 \text{ ml/min/1.73 m}^2$
women : $118 \pm 16 \text{ ml/min/1.73 m}^2$ or no significant difference


2-17 years: GFR= $110 \pm 17 \text{ mL/min/1.73 m}^2$


adults: GFR= $103 \pm 16 \text{ mL/min/1.73 m}^2$
INTERPRETATION: : normal values

in elderly people: - 1 ml/min/1.73 m² / yr
on the average (much scatter)

pregnancy: + 30%

after eating meat
or after dopamin-hyperamine infusion: + 15-20% (glomerular reserve)

nycthemeral cycle: increased during the day

after nephrectomy: progressive recovery (50→70%)
(in ca. 6 months)
INTERPRETATION: normal values

Lines - Historical normal range
Points – Grewal et al, 2005 Nucl Med Comm
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Practical issues in measurement

Choice of Method

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  normal values
  body size scaling
  case of children
normal GFR is supposed to be proportionnal to BSA

to determine renal function: YES!
GFR in mL/min./1.73 m²

to adapt a medication dosage: NO!
GFR in mL/min
(the absolute clearance is what matters
to clear the drug)

Dubois – Arch. Int. Med. 1916 ;17 :863

Improved formulæ: Haycock…

$$BSA_{m^2} = 7.184 \times 10^{-3} \times Height_{cm}^{0.725} \times Weight_{kg}^{0.425}$$
INTERPRETATION: scaling

Scaling **must** be done to assess the degree of renal function.
Scaling **must not** be done to determine high toxicity drug dosage.

**Example:** a GFR of 40 mL/min

- In an adult of 1.73 m² BSA:
  - 168 cm – 63 kg
  - nGFR = 40 mL/min/1.73 m²
  - Is abnormal (stage 3)
  - Carboplatin scaled to 40 mL/min

- In a child of 0.56 m² BSA:
  - 90 cm – 12 kg
  - nGFR = 123 mL/min/1.73 m²
  - Is normal
  - Not to 123 mL/min (adult dosage)
Scaling to ECV was proposed, reducing the equations to a slope-only technique.

This in only an approximation and remains quite controversial.

*Peters - Nephrol Dial Transplant 1992; 7: 205*
Clinical indications for renal clearance measurements

The concept of renal clearance

- renal function
- clearance
- glomerular

Methods of clearance measurements

- tracers
- types of clearances
- urinary clearance
- continuous infusion plasma clearance
- single shot plasma clearance
- normalisation for body size

Practical issues in measurement

Choice of Method

Interpretation

- normal values
- body size scaling
- case of children
INTERPRETATION: children

Piepsz, Eur. J. nucl. med. mol. imag. 2006; 33:1477
INTERPRETATION: children

normal GFR (2-15 years): 104 ± 20 mL/min/1.73 m²
(normal range ~ 64 – 144 mL/min/1.73 m²)

0 months  52 ± 9 mL/min/1.73 m²
1-4 months  62 ± 14 mL/min/1.73 m²
4-8 months  72 ± 14 mL/min/1.73 m²
8-12 months  83 ± 17 mL/min/1.73 m²
12-18 months  92 ± 18 mL/min/1.73 m²
18-24 months  95 ± 18 mL/min/1.73 m²

considered as renal maturation
(or would is just be a scaling artefact?)
To conclude…

routine technique = plasma creatinine with MDRD/Schwartz formula but:
- poor technique to detect renal diseases at early stage
- many assay techniques, with different results
- not precise (± 30 mL/min/1.73 m²)
- not adapted to all patients (obese, diabetic, liver disease…)

Poggio
To conclude…

Camera-based methods without blood sampling are not more precise. They must be used for relative renal function assessment.

DTPA/EDTA Clearance measurements* are precise (± 5 mL/min/1.73 m²). High care must be taken at all stages of the technique to get such a precision.

Radiation burden is very low (~ 20 μSv for EDTA).

Cold iohexol clearance is a second-hand choice.

* reference technique is bi-exponential
Question 1

What is the best index for renal function?  
(one choice only)

A – renal size

B – urine flow

C – renal blood/plasma flow

D – glomerular filtration rate

E – filtration fraction
Question 2

What is the best technique to assess glomerular filtration rate? (one choice only)

A – serum creatinine assay
B – MDRD formula
C – blood urea nitrogen
D – 99mTc-DMSA absolute uptake
E – plasma clearance of $^{51}$Cr-EDTA
Question 3

Should we normalise GFR to body size?
(one choice only)

A – no, never
B – yes, always, to body surface area
C – yes, most of the time, to body surface area
D – yes, always, to body weight
E – yes, most of the time, to body weight
Question 4

What could best characterise urine clearance measurements, as compared to plasma clearance measurements? *(one choice only)*

A – they are more precise and more accurate
B – they are less precise but more accurate
C – they are more precise but less accurate
D – they are less precise and less accurate
E – they are more precise and as accurate
Question 5

In which circumstance(s) should you perform a urinary clearance measurement instead of a plasma clearance measurement? *(potentially several answers)*

A – in children

B – in patients with oedema

C – in patients with hyperfiltration

D – in patients with very low renal function

E – in patients with asymmetrical renal function
Question 6

What is the general formula for clearance?
P: plasma concentration – U: urinary concentration
(one choice only)

A – P × U / V
B – U × V / P
C – U × V / BW
D – (C × O / V ) × (P / U)
E – P × V × U
Question 7

How is the plasma clearance determined? (one choice only)

A – the urinary concentration divided by the plasma concentration
B – the area under the plasma time-concentration curve divided by the injected activity
C – the area under the plasma time-concentration curve divided by the plasma concentration at time 0
D – the injected activity divided by the area under the plasma time-concentration curve
E – the injected activity divided by the area under the plasma time-concentration curve
Question 8

When using two plasma samples for plasma clearance technique:
(potentially several answers)

A – two exponentials must be determined

B – one exponential only can be determined

C – a correction formula must be used, the recommended one being published by Brochner and Mortensen

D – a correction formula must be used, the recommended one being published by Christensen and Groth

E – there is no need for correction
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What is the best index for renal function?  
(one choice only)

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E – filtration fraction
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