CB-2-C-2: Perform the analyses to laboratory standard operating procedures on patients with disorders of:

- ***Renal pathophysiology***

Acute kidney injury (AKI):
AKI is the sudden loss of proper kidney function over a short period of time, namely the previous 7 days. The causes of AKI are numerous but can generally be organised into 3 groups; pre-renal, intrinsic and post-renal. AKI is diagnosed using standard kidney function tests including blood urea and creatinine and urine output. AKI carries a range of potential complications including metabolic acidosis, elevated blood potassium, changes in fluid balance and potentially severe detrimental impact of other organ systems. Management involves treatment of underlying causes as well as supportive care, for example renal replacement therapy. Generally, both kidneys must be affected to cause AKI as normal function of one kidney is usually sufficient.

Pre-renal causes:
Pre-renal causes are those that decrease the effective blood flow to the kidneys which may result in renal ischaemia and depression of glomerular filtration. These include systemic causes, for example low blood volume, low blood pressure and heart failure as well as local changes to the blood vessels supplying the kidney including renal artery stenosis.

Intrinsic causes:
AKI resulting from damage to the kidney itself is called intrinsic AKI and can be due to damage to many structures including the glomeruli and renal tubules. Common causes of each are glomerulonephritis and acute tubular necrosis respectively. Other causes include rhabdomyolysis, tumour lysis syndrome, drugs and autoimmune conditions.

Post-renal causes:
Post-renal AKI is a consequence of urinary tract obstruction. This may be related to a number of conditions including prostatic hyperplasia, kidney stones, obstructed urinary catheter and bladder, ureteral or renal malignancy.

Guidelines for the diagnosis of AKI state that the following criteria should be used:

AKI stage:	Serum creatinine:	Urine output:
Stage 1	1.5 – 2 x baseline	<0.5 ml/kg/hr for 6hrs
Stage 2	2 – 3 x baseline	<0.5 ml/kg/hr for 12hrs
Stage 3	>3 x baseline *or* acute rise >50 µmol/L where baseline >300 *or* requirement for RRT	<0.3 ml/kg/hr for 24hrs *or* anuria for 12hrs

RRT – Renal replacement therapy

Chronic kidney disease (CKD):
CKD is the progressive loss of renal function, usually over a long period of months or years, and is distinct from AKI in that kidney impairment must last for over 3 months. CKD is diagnosed and monitored using a range of blood and urine tests and by measuring or estimating glomerular filtration rate. Renal biopsy and imaging can also be used to investigate and characterise kidney damage.
One of the most commonly recognised causes of CKD is diabetes mellitus. Other prominent causes include idiopathic CKD, hypertension, and glomerulonephritis. Together, these cause approximately 75% of all adult cases. Other causes include vascular disease such as

bilateral renal artery stenosis and ischemic nephropathy, glomerular disease such as and IgA nephropathy and diabetic nephropathy, congenital disease such as polycystic kidney disease and obstructive nephropathy such as kidney stones and diseases of the prostate.

CKD is often classified into 5 stages, as shown below, depending on the severity of the condition:

CKD Stage:	Description:	GFR (ml/min/1.73m^2):
Normal kidney function	Normal kidneys	≥90
1	Kidney damage and normal GFR	≥90
2	Kidney damage and mild decrease in GFR	60 – 89
3a	Mild to moderate decrease in GFR	45 – 59
3b	Moderate decrease in GFR	30 – 44
4	Severe decrease in GFR	15 – 29
5	Established renal failure	<15 or on dialysis

All individuals with a GFR <60 for 3 months are classified as having CKD irrespective of the presence or absence of kidney damage. A reduction in kidney function to this level or lower represents loss of half or more of normal adult level kidney function. GFR can be estimated (eGFR) by using different equations and a patient's serum creatinine value. One such equation is the MDRD equation shown below:

$$eGFR\ (ml/min/1.73m^2) = 175 \times ((plasma\ creatinine\ (umol/l)/88.4)^{-1.154}) \times age\ (years)^{-0.203}$$

Results be multiplied by 0.742 for females and 1.21 for African Americans.

Protein in the urine is classed as an indicator of kidney disease and the letter "P" is appended after the stage if protein loss is significant. Albumin / creatinine ratio (ACR) measurement is the recommended first line test for proteinuria by NICE as it has greater sensitivity for detecting low levels of proteinuria. In those with established renal disease, protein / creatinine ratio (PCR) may be used instead of ACR to quantify and monitor greater levels of proteinuria. The presence of protein in the urine is a strong prognostic indicator of the likelihood of kidney disease progression.
In diabetics, an ACR >2.5 mg/mmol in men and >3.5 mg/mmol in women is considered significant. In non-diabetics, an ACR >30mg/mmol is considered significant. If an initial sample ACR is >30 but <70 then this must be confirmed on a second early morning sample. ACRs >70 do not need to be repeated. Patients should be tested for proteinuria if they have any of the following risk factors:

- GFR <60
- Diabetes
- Hypertension
- Cardiovascular disease
- Structural renal tract disease, multiple renal calculi or prostatic hypertrophy
- Multisystem diseases with potential kidney involvement
- Family history of CKD stage 5 or hereditary kidney disease
- Haematuria

ACR can be combined with CKD stage in order to jointly classify and assess CKD associated risk, as shown below:

		ACR:		
		<3	3 – 30	>30
GFR:	CKD stage:	A1	A2	A3
≥90	1	No CKD in absence of markers of kidney damage		
60 – 89	2			
45 – 59	3a			
30 – 44	3b			
15 – 29	4			
<15	5			

Increasing risk →

Increasing risk →

		ACR:		
		<3	3 – 30	>30
GFR:	CKD stage:	A1	A2	A3
≥90	1	≤1	1	≥1
60 – 89	2	≤1	1	≥1
45 – 59	3a	1	1	2
30 – 44	3b	≤2	2	≥2
15 – 29	4	2	2	3
<15	5	4	≥4	≥4

Left – Tables showing increasing risk of adverse events using a combination of CKD staging and ACR
Right – Number of times per year GFR should be measured according to combined CKD stage and ACR

ACR requires the measurement of both creatinine and albumin. Creatinine is measured using an enzymatic method as detailed below:

$$\text{Creatinine} + H_2O \xrightarrow{\textit{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\textit{Creatinase}} \text{Sarcosine} + \text{urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\textit{Sarcosine oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{HTIB} \xrightarrow{\textit{Peroxidase}} \text{Quinone imine chromogen} + H_2O + HI$$

The colour intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration in the original sample and is measured at 546nm.

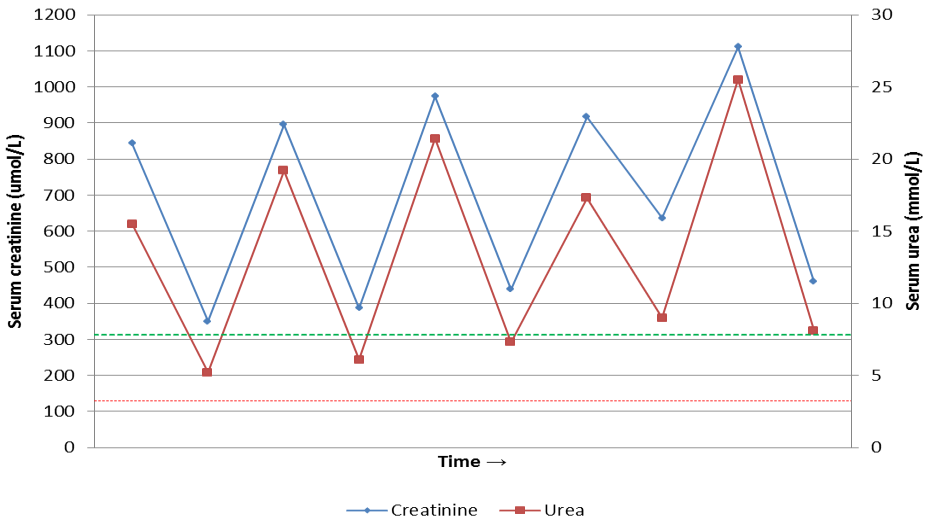
Albumin is measured using a colourimetric assay shown below:

$$\text{Albumin} + \text{BCG} \xrightarrow{\textit{pH 4.1}} \text{Albumin-BCG complex}$$

At a pH 4.1 albumin displays a cationic character enabling it to bind with the anionic dye bromcresol green to form a blue/green complex. The intensity of this complex is directly proportional to the albumin concentration in the original sample and is measured photometrically at 570nm.

Dialysis:
Dialysis is a mechanical process used to mimic the filtration action of the kidneys in order to clean waste substances from the blood of patients with renal failure. Dialysis works on the

principles of the diffusion of solutes and ultrafiltration of fluid across a semi-permeable membrane. A semi-permeable membrane contains pores of various sizes which allow smaller solutes and fluid to pass through but block larger substances, for example red blood cells and large proteins, replicating what happens in the glomerulus. This membrane separates dialysate and blood which flow past each other in opposite directions to maximise the concentration gradient for those solutes which need to be filtered and are high in concentration in the blood and low in the dialysate. To aid in this process, the dialysate is constantly replaced. The dialysis solution has solutes such as potassium and calcium in similar levels to their natural concentration in healthy blood. For bicarbonate, dialysis solution is usually set at a slightly higher level than in normal blood to encourage diffusion of bicarbonate into the blood. This acts as a pH buffer to neutralise the metabolic acidosis that is often present in these patients. The levels of the components of dialysate are typically prescribed by a nephrologist according to the needs of the individual patient. Patients will have routine U+E blood tests performed pre and post-dialysis to monitor their blood electrolyte and creatinine levels and to see how effective the procedure has been. Pre-dialysis samples can also be used to decide on the type and contents of the dialysis solution to be used. The blood results of patients on dialysis commonly show a characteristic pattern of rising and falling creatinine and urea pre and post-dialysis respectively, shown in the graph below:



Creatinine upper limit of normal (130 µmol/L) shown my red dotted line
Urea upper limit of normal (7.8 mmol/L) shown by green dotted line

The concentration of potassium in the blood is also monitored and shows a similar pattern of rises and falls pre and post-dialysis. It is important to monitor potassium as high levels can cause dangerous cardiac arrhythmias.

Renal transplant:
Kidney transplants involve the surgical removal of a kidney from a living or deceased donor which is then grafted into the circulation of a recipient patient. The donor and recipient must both be a close match in terms of tissue and blood type in order to minimise the risk of transplant rejection. The recipient must also take immunosuppressive drugs for as long as they have the transplanted organ to avoid rejection. Commonly used immunosuppressant drugs include tacrolimus and cyclosporin which both reduce T cell activity and therefore the immune response. When a T cell receptor is bound by a complimentary antigen, a signalling cascade is activated which increases intracellular calcium and activates calcineurin.

Calcineurin dephosphorylates NF-AT which promotes expression of genes coding for IL-2 and related cytokines. Tacrolimus and cyclosporin both inhibit calcineurin dephosphorylation of NF-AT and as such IL-2 transcription and T cell activation.
The concentrations of immunosuppressant drugs are routinely monitored in patient's blood to ensure that they are within the therapeutic range and avoid the complications of toxicity or organ rejection. This allows for the specific tailoring of a patients dose to what they required. Measurement is performed using a LC-MS/MS assay which simultaneously detects and quantifies both drugs.

Drug:	Therapeutic range (µg/L):	Collection time:	Frequency of monitoring:
Tacrolimus	10 – 12 8 – 10 (>6 months post-transplant)	Pre-dose	Initially 3 x per week Less when stable
Cyclosporin	150 – 275 100 – 200 (stable graft)	Pre-dose	Initially 3 x per week Less when stable

Renal tubular acidosis (RTA):
Type 1 – Distal RTA:
Distal RTA is characterised by a failure of H^+ secretion into the lumen by the alpha intercalated cells of the medullary collecting duct of the distal nephron. This leads to an inability to acidify the urine to a pH of less than 5.5. Because renal excretion is the primary means of eliminating H^+ from the body there is a tendency towards acidemia. There is also an inability to reclaim potassium as this is facilitated by the H^+/K^+ antiporter causing hypokalemia. Increased aldosterone also causes potassium loss. Additionally, calcium phosphate stones demonstrate a tendency for deposition at higher pHs causing the kidney to develop stones bilaterally; this does not occur in the other RTA types. Increased urine calcium is caused by increased resorption of calcium phosphate from the bones in an attempt to buffer excess acid. Type 1 RTA is characterised by:

- Normal anion gap metabolic acidosis
- Hypokalemia, hyperchloremia, hypercalciuria, low urine citrate
- Urinary stone formation
- Nephrocalcinosis
- Bone demineralisation

The diagnosis of distal RTA can be made by the observation of a urinary pH >5.5 in the face of a systemic acidemia. Failure to acidify the urine following an oral acid challenge is often used as a test, using ammonium chloride tablets as an acid load. An alternative test involves the simultaneous administration of furosemide and fludrocortisone to increase distal tubular sodium delivery, principal cell sodium reabsorption, and alpha-intercalated cell proton secretion. Again, a failure to acidify the urine <5.5 would suggest distal RTA.

Type 2 – Proximal RTA:
Proximal RTA is caused by a failure of the proximal tubular cells to reabsorb filtered bicarbonate from the urine, leading to urinary bicarbonate wasting and acidemia. The distal intercalated cells function normally, so the acidemia is less severe than distal RTA and the urine can acidify to a pH of <5.5. Proximal RTA is usually associated with a more generalised dysfunction of the proximal tubular cells called Fanconi syndrome which also includes defects in the absorption of glucose, amino acids, phosphate, uric acid, and other organic anions such as citrate.
Approximately 90% of filtered HCO_3^- is absorbed by the proximal tubule, the rest is absorbed by the distal nephrons. In proximal impairment of HCO_3^- reabsorption, the distal nephrons become overwhelmed by increased HCO_3^- and cannot compensate for the loss in proximal function. As urinary HCO_3^- loss progresses, plasma HCO_3^- drops to 15-18 meq/L. This causes the level of filtered HCO_3^- to fall and thus there is reduced delivery of HCO_3^- to the distal

nephrons. The distal nephrons are no longer overwhelmed and can regain function, leading to a reduction in urinary bicarbonate loss and a urine which can now be acidic. Urinary K^+ wasting and hypokalemia are common due to persistent hyperaldosteronism. Hyperaldosteronism is related to the defect in proximal reabsorption of filtered HCO_3^- which leads to decreased proximal NaCl reabsorption and a tendency for salt wasting. The factors responsible for the defects in proximal transport are incompletely understood. There are three features of the proximal tubules that are vital to proximal reabsorption of HCO_3^-:

- The Na^+/H^+ exchanger in the luminal membrane.
- The Na^+/K^+ ATPase pump in the basolateral membrane.
- Carbonic anhydrase, located both intracellularly where it results in the generation of H^+ and HCO_3^- and in the lumen where it facilitates HCO_3^- reabsorption.

It has been proposed that one or more of these factors must be impaired to account for the defect in distal RTA.

Type 3 – Combined proximal and distal RTA:
In some patients, RTA shares features of both distal RTA and proximal RTA and may be observed as the result of inherited carbonic anhydrase II deficiency. Mutations in the gene encoding this enzyme give rise to an autosomal recessive syndrome of osteopetrosis, renal tubular acidosis, cerebral calcification, and mental retardation.

Type 4 RTA:
Type 4 RTA is due either to a deficiency of aldosterone or to a resistance to its effects and is associated with a mild normal anion gap metabolic acidosis. This is due to a physiological reduction in proximal tubular ammonium excretion secondary to hypoaldosteronism and results in a decrease in urine buffering capacity. Its cardinal feature is hyperkalemia. Urinary acidification is normal, hence it is often called hyperkalemic RTA or tubular hyperkalemia. Causes include:

- Primary or secondary hypoaldosteronism
- Aldosterone resistance
- Drugs including NSAIDs, ACE inhibitors, ARBs and spironolactone

	Type 1	Type 2	Type 4
Basic defect	Decreased distal acidification	Decreased proximal HCO_3^- reabsorption	Aldosterone deficiency or resistance
Normal anion gap metabolic acidosis	Yes	Yes	Yes
Urine anion gap	Positive	Positive	Positive
Minimum urine pH	>5.5	Variable, >5.5 if given alkali load	<5.5
Serum K^+	Low	Low	High
% filtered HCO_3^- excreted	<10	>15	<10
Plasma HCO_3^-	May be <10	Usually 14 – 20	Usually >15
Acid excretion	Low	Normal	Low
Stones/nephrocalcinosis	Yes	No	No
Franconi syndrome	No	Yes	No

Renal calculi:

Renal stones are solid pieces of material formed in the kidneys from various minerals and components of urine. They are typically passed from the body along with urine and if small in size my cause no problems and pass largely unnoticed. If they growth to a significant size they can potentially cause blockage of the urethra leading to severe pain in the flank and lower back and spreading to the groin. This is known as renal colic and typically occurs in waves lasting 20 to 60 minutes. Other symptoms may include nausea, fever, haematuria, pus in the urine and pain on urination. Blockage of the urethra can cause a decrease in kidney function.

Risk factors for stone formation include obesity, dehydration or low fluid intake and high dietary animal protein, salt, refined sugars, oxalate and some fruit juices. Predisposition to stone formation is also caused by some metabolic conditions such as distal RTA and hyperparathyroidism. A patient with recurrent kidney stones may be screened for such disorders, typically with a 24 hour urine collection analysed for features that promote stone formation. In those who have previously had stones, prevention is recommended by drinking fluids such that more than two liters of urine is produced per day whilst avoiding soft drinks containing phosphoric acid. If this is not effective, thiazide diuretics may be given. Stones are typically classified by their location in the kidney (nephrolithiasis,) ureter (ureterolithiasis), or bladder (cystolithiasis), or by their chemical composition. When a stone causes no symptoms, no treatment is needed. For stones which are causing symptoms, pain control is usually the first measure using NSAIDs and opioids. More severe cases may require intervention, for example extracorporeal shock wave lithotripsy to break larger stones into smaller fragments, or cystoscopic procedures.

Stone type:	Population:	Circumstances:	Colour:
Calcium oxalate	*80%*	*Acidic urine*	*Black / dark brown*
Factors promoting the precipitation of oxalate crystals produce hyperoxaluria. Oxaluria is increased in certain GI disorders including IBD, in patients with resection of the small bowel or small bowel bypass and patients who consume increased amounts of oxalate. Primary hyperoxaluria is a rare autosomal recessive condition which usually presents in childhood.			
Calcium phosphate	*5 – 10%*	*Alkaline urine*	*Dirty white*
The formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and distal RTA.			
Uric acid	*5 – 10%*	*Persistently acidic urine*	*Yellow / red brown*
People with certain metabolic abnormalities may produce uric acid stones. They also may form in association with hyperuricosuria with or without hyperuricemia and acid/base disorders where the urine is excessively acidic. Urate stones are especially common after colon resection. Patients can be treated with allopurinol which will reduce urate formation. Urine alkalinization may also be helpful.			
Struvite	*10 – 15%*	*Kidney infections*	*Dirty white*
Struvite (magnesium ammonium phosphate) crystals form most often in the presence of urea-splitting bacteria which metabolise urea to ammonia and CO_2. *Proteus mirabilis, Proteus vulgaris*, and *Morganella morganii* are the most commonly associated organisms. Commonly observed in those with factors predisposing them to UTIs.			
Cystine	*1 – 2%*	*Genetic disorder*	*Pink / yellow*
Patients with cystinuria, cystinosis, and Fanconi syndrome may form stones composed of cystine. Cystine stone formation can be treated with urine alkalinisation and dietary protein restriction.			
Xanthine	*Extremely rare*		*Brick red*
Patients with xanthinuria often produce stones composed of xanthine.			

Passed stones can be collected and analysed via infrared or X-ray crystallography to determine their chemical composition and give clues to the cause of their formation.

NICE CG 182:
Updates to NICE guidance recommends that laboratories should now use the CKD-EPI equation for estimation of GRF using enzymatic creatinine assays with calibration traceable to standardised reference material. The CKD-EPI equation is shown below:

GFR = 141 X min(Scr/κ,1)α X max(Scr/κ,1)-1.209 X 0.993Age X 1.018 [if female] X 1.159 [if black]

Scr = serum creatinine (mg/dL)
κ = 0.7 (female), 0.9 (male)
α = –0.329 (female), –0.411 (males)

The CKD-EPI equation was developed in an effort to create a formula more precise than the MDRD formula and has less bias and greater accuracy than the MDRD equation especially at higher GFRs.

NICE also recommends the use of eGFR based on cystatin C measurement to confirm or rule out CKD in people with eGFR based on creatinine 45-59 for ≥90 days, no proteinuria (ACR <3) and no other markers of kidney disease. Cystatin C is a 120 amino acid protein encoded by the CST3 gene and produced by all nucleated cells. Creatinine is inaccurate at detecting mild renal impairment and levels can vary with muscle mass and protein intake. Cystatin C is less dependent on age, sex, race and muscle mass compared to creatinine. As kidney function and glomerular filtration rate decline the amount of cystatin C in the blood will rise.