Diagnostic testing for urology cases

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Urology
- from Greek οὖρον - ωύρον, "urine" and -λογία, -logia "study of" is the medical and surgical specialty that focuses on the urinary tracts of males and females

Testing for urology cases
- Urine (± urine culture)
- Routine blood tests
- GFR measurement
- Diagnostic imaging
  - Radiographs (± contrast)
  - Ultrasound
  - Endoscopy
  - MRI / CT
- Biopsy

Urine collection
- Mid-stream voiding (cats: non-absorbable litter)
- Contamination from urethra etc.
- Not advised for culture
- Good to test for haematuria
- Manual bladder expression
- Use mainly in anaesthetised patients
- Catheterisation
  - Male dog: pre-measure length, no force; good for all purposes including urine culture
  - Female dog: dorsal recumbency, use speculum
  - Tomcats: extend penis caudally
  - Female cats: anaesthesia required, ventral recumbency

Urine collection
- Cystocentesis
  - Under palpation, blind, under ultrasound control
  - Advantage: no contamination, easy to perform, well tolerated
  - Disadvantage: may introduce micro-haematuria, puncture other organs, dissemination of transitional cell carcinoma (?)
  - Contraindication: <20'000 PLT/µl

Urinalysis
- Perform as quickly as possible after collection (<30 min)
- Use minimum of 3 ml urine
- If refrigerated – warm before analysis
- Record type of collection
  - Colour and transparency
  - Specific gravity (refractometer)
  - Dip-stick analysis
  - Sediment
  - Bacterial culture
  - Others
Physical properties

- Urine colour
  - Derived from urochromes
- Transparency
  - Normally clear
  - Cloudy: mostly WBC, casts, crystals
- Odor
  - Ammonia odor due to bacteria

Urine specific gravity: 3 methods

- Only refractometer gives accurate results (never use dipstick value)
- Measures total number of solutes and their weight
  - 1000 mg/dl (55 mmol/l) glucose increases USG by 0.004
  - 1000 mg/dl protein increased USG by 0.003
- Measure USG BEFORE any fluid or drugs are given

Chemical properties

- pH
- Protein
- Glucose
- Ketones
- Bilirubin
- Occult blood
- USG, leucocytes, urobilinogen, others not useful in dogs and cats

- Warm urine after refrigeration
- Use centrifuged urine
- Use proper timing: normally 1 minute after drop has been applied
- Keep strips air-tight
- Avoid desinfectant
Urine pH
- Normally measured on dip-stick but ok with any pH-meter
- Normal: 5-7.5
- Varies based on diet and acid-base status
- Plant based diet causes more alkaline urine
- Cats have post-prandial alkaline tide
- UTI with urease producing bacteria have alkaline urine

Protein in urine
- On dip-stick: negative, + (30mg/dl), ++ (100mg/dl), +++ (1000mg/dl)
- Always assess protein in relation to USG (see UPC)
- False positive in very alkaline, false negative in very acidic urine
- Urine on dipstick is commonly albumin
- Categorise into pre-renal, renal and post-renal
- Always assess protein in relation to sediment result
  - Pre-renal: Bence-Jone Protein, haemoglobin
  - Post-renal: UTI, neoplasia, genital disease

Urin Protein-Creatinine ratio (UPC)
- Protein on dipstick dependent on urine SG
- To assess urine protein excretion one needs metabolic cage
- UPC and 24-h protein excretion are very closely correlated
- Normal: < 0.2 (dogs and cats) (see IRIS guidelines)
- Measure UPC only with inactive sediment!

Glucose in urine
- Normally negative in dogs and cats (all glucose reabsorbed in proximal tubule)
- Positive if serum glucose > renal threshold
  - Dogs >180 mg/dl = 10 mmol/l
  - Cats >240-300 mg/dl = 13-16 mmol/l
- False positives: rarely with some drugs (antibiotics)

Occult blood in urine
- Detects RBC, haemoglobin and myoglobin
- Interpretation: together with sediment analysis and plasma colour

Haematuria
- Trauma, urolithiasis, tumour, infection, inflammation, coagulopathy, etc.
- Haemoglobinuria
- Haemolysis (mostly intravascular)
- Myoglobinuria
- Crush injury, heat stroke, seizure, greyhound myopathy, severe hypokalaemia (cats)
Others
- Ketones
  - Acetoacetate, acetone in urine give positive results, β-Hydroxybuturate (other breakdown of fatty oxidation) does not
  - Most commonly in diabetic ketoacidosis or diabetic ketosis
- Bilirubin
  - From breakdown of excessive haemoglobin (only conjugated bilirubin in urine)
  - In dogs + (to ++ in males) of bilirubin can be normal
  - In cats bilirubin is always abnormal
- Cases: haemolysis, liver disease, post-hepatic disease, fever, starvation

Sediment
- Must be done on fresh urine samples (casts and crystals might otherwise disappear)
- Centrifugation: 1000-1500 rpm for 5 minutes
- Interpretation: knowledge of USG and way of collection
- Record all findings and amount (neg, +, ++, +++)
  - Casts on 10x (LPF)
  - Cells and bacteria on 40x (HPF)
  - Crystals (type)
  - Others (fat droplet, sperm, artefacts and contaminants)

Cells
- Red blood cells:
  - Numbers depend on collection technique (commonly < 5RBC / HPF)
  - Can come from entire urogenital tract
  - Causes: see lecture abnormal urine colour
- White blood cells:
  - Numbers depend on collection (more in voided samples) (commonly < 5WBC / HPF)
  - Increased numbers = pyuria
  - Indication of inflammation in urinary or genital tract (localisation unclear). Most often UTI

Cells
- Squamous epithelial cells:
  - Common in voided and catheter samples
  - No significance
- Transitional epithelial cells:
  - Arise from renal pelvis to urethra
  - Seen in normal (few, during sampling) and abnormal samples (inflammation, trauma, FLUTD, etc.)
  - May become malignant ➔ diagnosis of TCC
- Renal epithelial cells:
  - Are always abnormal (ischemia, inflammation, AKI, etc.)

Casts
- Cylindrical moulds from lining of tubules with cells and/or inflammatory proteins (Tamm Horsefall mucoprotein)

Organisms
- Urine is sterile. Collection method important
- Pyuria most often together with bacteria (exceptions)
- UTI also possible without visible bacteria
- Parasite eggs:
  - Dioctophyma renale
  - Capillaria spp.
**Crystals**
- Many crystals are normal in dogs and cats (struvite, calcium-oxalate)!
- Crystalluria ≠ Urolithiasis!!
- Crystals may dissolve or form de novo (especially during refrigeration)
- Crystal formation depends on pH, temperature und osmolality

**Urinary biomarkers**
- Should demonstrate kidney injury early
- Should also show localisation of injury (Glomerulus, Tubules, both)
- Differentiate renal from pre-renal/post-renal
- Assess severity of injury
  - Proteins are normally not in urine and can be used as biomarkers

**Potential urinary protein biomarker in dogs**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Disease with increased values</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Molecular Weight proteins</td>
<td></td>
</tr>
<tr>
<td>- Albumin</td>
<td>Chronic kidney disease (CKD)</td>
</tr>
<tr>
<td>- Immunoglobulin G</td>
<td>Leishmaniasis, Leptospirosis, Pyometra</td>
</tr>
<tr>
<td>- Immunoglobulin A</td>
<td>Leishmaniasis, Leptospirosis</td>
</tr>
<tr>
<td>Low Molecular Weight Proteins</td>
<td></td>
</tr>
<tr>
<td>- Retinol-binding protein</td>
<td>CNE, urolithiasis</td>
</tr>
<tr>
<td>- α- and β-Microglobulin</td>
<td>α-linked hereditary nephropathy</td>
</tr>
<tr>
<td>Tubular enzymes</td>
<td></td>
</tr>
<tr>
<td>- N-acetyl-β-D-glucosaminidase</td>
<td></td>
</tr>
<tr>
<td>- γ-Glutamyltransferase</td>
<td>Pyometra, AKI</td>
</tr>
<tr>
<td>Inflammatory proteins</td>
<td></td>
</tr>
<tr>
<td>- Interleukin-α, -β</td>
<td>Cisplatin-induced AKI</td>
</tr>
<tr>
<td>- Neutrophil gelatinase associated lipocalin (NGAL)</td>
<td>AKI versus CKD, others</td>
</tr>
</tbody>
</table>

**Routine blood tests**
- Urea (BUN)
- Creatinine
- Phosphorus
- Total protein
  - Albumin / Globulin
- Electrolytes
- Cholesterol
Azotaemia etc.
• Azotaemia = Increase in urea and creatinin
  • Pre-renal azotaemia (decreased perfusion of kidney, hypovolaemia, anaemia, heart failure, etc.)
  • Renal azotaemia (intrinsic kidney disease
  • Post-renal azotaemia (obstruction of urine flow irrespective where)
• Uraemia = syndrom of azotaemia with clinical signs (anorexia, vomiting, ulcers, bad breath, etc.)

Azotaemia
\[
\begin{align*}
\text{Urea} & \uparrow \text{ and Creatinine} \uparrow \\
\text{Pre-renal} & \quad \text{Renal} & \quad \text{Post-renal} \\
\text{USG} & \uparrow & \text{USG} \downarrow \\
\text{Urea:creatinin-ratio does not differentiate}
\end{align*}
\]

Urea
\[
\begin{align*}
\text{Colon} & \quad \text{Body proteins} \\
\text{Food Proteins} & \downarrow \text{NH}_3 \\
\text{NH}_3 & \uparrow \text{Urea cycle} \\
\text{Liver} & \quad \text{Urea} \\
\text{Kidney} & \downarrow \text{Urea} \\
\text{Faeces} & \downarrow \text{25%} \\
\text{Urea} & \quad \text{Urine} \quad \text{75%} \\
\text{Creatinine} & \quad \text{2%} \text{ daily break down}
\end{align*}
\]

Abnormal urea
Increase
• Post-prandial (4-6 hours after protein meal)
• Gastrointestinal bleeding
• Increased catabolism (infection, fever, ...)
• Certain drugs (steroids)
Decrease
• Low protein diet
• Polyuria
• Hepatopathy, most commonly portosystemic shunt
• Anabolic steroids

Creatinine
\[
\begin{align*}
\text{Muscle} & \quad \text{Creatine} \\
\text{Kidney} & \quad \text{Creatinine} \\
\text{Creatinine} & \quad \text{Freely filtered} \\
\text{Creatinine} & \quad \text{Not reabsorbed} \\
\text{Creatinine} & \quad \text{Secreted} \quad \text{-> 5%}
\end{align*}
\]

Osborne & Finco: Canine and Feline Nephrology and Urology
Discordant Results between urea and creatinine

<table>
<thead>
<tr>
<th>Disproportionate ↑ urea relative to creatinine</th>
<th>Disproportionate ↑ creatinine relative to urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe dehydration or volume depletion (common)</td>
<td>Liver diseases</td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>Anorexia or low protein diet</td>
</tr>
<tr>
<td>Emaciated animal</td>
<td>Massive muscle injury (acute)</td>
</tr>
<tr>
<td>Young animal</td>
<td>Well-muscled animal</td>
</tr>
</tbody>
</table>

- Discordant results can not always be explained

Important facts

- NORMAL UREA AND CREATININE DOES NOT NECESSARILY IMPLY NORMAL KIDNEY FUNCTION
- INCREASE IN CREATININE OVER TIME MAY POINT TOWARDS PROGRESSIVE LOSS OF RENAL FUNCTION EVEN IF VALUES ARE STILL WITHIN NORMAL LIMITS

Routine blood tests

- Phosphorus
- Total protein
  - Albumin / Globulin
- Electrolytes
- Cholesterol

- Phosphorus: filtered in glomeruli and reabsorbed in tubulus. >85% nephron loss to see increased phosphorus
- Albumin normally not excreted via kidney; may be lost in PLN
- Cholesterol increases in animals with nephrotic syndrome
Serum renal biomarkers
- Cystatin C
- NGAL
- Homocystein
- Big-endothelin-1

Glomerular filtration rate (GFR)
- Definition:
  - Estimated clearance, defined as volume of plasma cleared of a given substance over time
  - Influenced by many non-renal factors (protein intake, hydration status, sodium balance, gender, age, breed, day-to-day variation, etc.)
- Wide reference interval of normal GFR in healthy animals

GFR measurement
- GFR can not be measured directly
- Renal clearance of substance (x) that is neither reabsorbed nor secreted by tubules = GFR
- \[ \text{GFR} = \frac{V \times C_{U}}{t \times C_{P}} = \text{ml/min} \]
  - \( C_{U} = \text{concentration in urine (mg/ml)} \)
  - \( V = \text{volume of urine (ml)} \)
  - \( t = \text{specific amount of time (min)} \)
  - \( C_{P} = \text{concentration in plasma (mg/ml)} \)

Ideal substance to measure GFR
- Freely filtered in glomerulum
- Not reabsorbed in tubulus
- Not secreted in tubulus
- Not protein bound
- Non-toxic
- Does not alter GFR
- Easily measured in plasma and urine
- \( \text{INULIN (fructose polymere)} \)

Clearance
- Calculation from plasma concentration versus time
  \( \rightarrow \) Clearance (\( C_{\text{plasma}} \))
- Calculation from plasma and urine data
  \( \rightarrow \) Clearance (\( C_{\text{renal}} \))
- If substance is excreted solely via kidney
  \( \rightarrow C_{\text{plasma}} = C_{\text{renal}} \)
- Without tubular secretion or reabsorption
  \( \rightarrow C_{\text{plasma}} = \text{GFR (e.g. Inulin – Clearance)} \)

Two types of Clearance
- Endogenous Clearance
  - Renal - Clearance
    \[ C_{\text{renal}} = \frac{V}{t} \times \frac{C_{U}}{C_{P}} \text{ (mL/min)} \]
- Exogenous Clearance
  - Plasma - Clearance
    \[ C_{\text{plasma}} = \frac{D}{AUC} \text{ (mL/min)} \]

\( D = \text{dose of marker;} \)
\( AUC = \text{area under plasma concentration versus time curve} \)
Glomerular filtration rate (GFR)
- Used substances
  - Renal Inulin clearance (Gold Standard)
  - Endogenous creatinine clearance
  - Exogenous creatinine clearance
  - Plasma Inulin clearance
  - Plasma iohexol clearance
  - Radioactive isotopes (± Scintigraphy)
  - Single injection without collection of urine possible

Renal inulin clearance
- Gold standard
- Inject inulin i.v. (bolus or constant rate infusion)
- Collect urine over 24 hours (metabolic cage)
- Measure inulin in plasma and urine
- Calculate GFR based on formula
- Very cumbersome, rarely performed (only research)