NEW TESTS FOR RENAL DISEASE: WHAT DO THEY MEAN?
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GENERAL KEY POINTS
Management of chronic kidney disease (CKD) and acute kidney injury (AKI) depends on diagnosis and on monitoring in order to make adjustments to therapy. There are several tests of renal function: some are measures of glomerular function while others are measures of tubular function. Many of these tests can be performed in practice providing invaluable information for patient care.

International Renal Insufficiency Society (IRIS) Staging
International Renal Insufficiency Society (http://www.IRIS-kidney.com) has developed staging system for animals with CKD and AKI and treatment based on staging. The CKD staging system is designed for use with dogs and cats after a diagnosis of CKD is made and staging is accomplished by evaluating

1. 2 serum creatinine values when patient is well hydrated,
2. 2 to 3 urine UPC and
3. 2 to 3 indirect arterial blood pressure determinations.

CKD is staged by magnitude of renal dysfunction and further modified (sub-staged) by presence or absence of proteinuria and/or hypertension. Proteinuria ONLY refers to renal proteinuria and not pre-renal (e.g. hyperglobulinemia) or post-renal (e.g. urinary tract infection, hematuria, etc), and is based on UPC. Blood pressure determination should be performed several times in order to account for a “white coat” effect using a standard protocol.

The AKI staging system is designed for use with dogs and cats who may have one or more of a spectrum of disease associated with a sudden onset of renal parenchymal injury most typically characterized by generalized failure of the kidneys to meet the excretory, metabolic, and endocrine demands of the body.

IRIS Grading of chronic kidney disease (CKD)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Plasma creatinine mg/dl</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dogs</td>
<td>Cats</td>
</tr>
<tr>
<td>1</td>
<td>&lt;1.4</td>
<td>&lt;1.6</td>
</tr>
<tr>
<td>2</td>
<td>1.4 - 2.0</td>
<td>1.6 - 2.8</td>
</tr>
<tr>
<td>3</td>
<td>2.1 - 5.0</td>
<td>2.9 – 5.0</td>
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<tr>
<td>4</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
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<table>
<thead>
<tr>
<th>UPC value</th>
<th>Substage</th>
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<tbody>
<tr>
<td>Dogs</td>
<td>Cats</td>
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<tr>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
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<tr>
<td>0.2 to 0.5</td>
<td>0.2 to 0.4</td>
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<tr>
<td>&gt;0.5</td>
<td>&gt;0.4</td>
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<tr>
<td>Systolic BP mm Hg</td>
<td>Diastolic BP mm Hg</td>
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<tr>
<td>------------------</td>
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</tr>
<tr>
<td>&lt;150</td>
<td>&lt;95</td>
</tr>
<tr>
<td>150 – 159</td>
<td>95 - 99</td>
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<tr>
<td>160 – 179</td>
<td>100 - 119</td>
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<tr>
<td>= 180</td>
<td>= 120</td>
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No evidence of end organ damage/complications | No complications (nc) |
Evidence of end organ damage/complications | Complications (c) |
Blood pressure not measured | Risk not determined (RND) |

**IRIS Grading of acute kidney injury (AKI)**

<table>
<thead>
<tr>
<th>AKI Grade</th>
<th>Blood creatinine</th>
<th>Clinical description</th>
</tr>
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<tbody>
<tr>
<td>Grade I</td>
<td>&lt; 1.6 mg/dl</td>
<td>Non Azotemic AKI:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Documented AKI:</td>
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<tr>
<td></td>
<td></td>
<td>(Historical, clinical, laboratory, or imaging evidence of acute</td>
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<tr>
<td></td>
<td></td>
<td>kidney injury, clinical oliguria/anuria, volume responsiveness†)… and/or</td>
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<tr>
<td>Grade II</td>
<td>1.7 – 2.5 mg/dl</td>
<td>Mild AKI:</td>
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<tr>
<td></td>
<td></td>
<td>a. Documented AKI and static or progressive azotemia</td>
</tr>
<tr>
<td>Grade III</td>
<td>2.6 – 5.0 mg/dl</td>
<td>Moderate to Severe AKI:</td>
</tr>
<tr>
<td>Grade IV</td>
<td>5.0 – 10.0 mg/dl</td>
<td>a. Documented AKI and increasing severities of azotemia and functional renal failure</td>
</tr>
<tr>
<td>Grade V</td>
<td>&gt; 10.0 mg/dl</td>
<td></td>
</tr>
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</table>

(†Volume responsive is an increase in urine production to >1 ml/kg/hr over 6 hours; and/or decrease in serum creatinine to baseline over 48 hours)

Each grade of AKI is further subgraded as:
1. Non oliguric (NO) or oligoanuric (O)
2. Requiring renal replacement therapy (RRT)

**NORMAL PHYSIOLOGY**

The nephron, the functional unit of the kidney, consists of a glomerular capillary network, a proximal convoluted tubule, the loop of Henle, a distal convoluted tubule, and a collecting duct. While renal function is often thought of in terms of azotemia, a reflection of glomerular function, tubular function is responsible for the final composition of urine through reabsorption and secretion of compounds (e.g. electrolytes, water). It is also involved in metabolism of hormones (e.g. erythropoietin, renin), and in maintaining systemic acid-base balance.
**TESTS OF RENAL FUNCTION**

**Tests of glomerular function**

GFR can be estimated using both clearance methods and “spot” or single time point tests. Renal or plasma clearance of an injected substance (e.g., iohexol, creatinine) is most accurate estimate of GFR. It is more sensitive means for detecting early CKD than spot methods of GFR estimation. Determining plasma clearance can be a relatively expensive and time-consuming procedure. It is most often performed to establish a decrease in GFR when clinical parameters (e.g., poorly concentrated urine) create suspicion for CKD but cannot confirm its presence, and to determine dosage regimens for therapeutic agents whose excretion is primarily renal in patients with CKD.

*Plasma clearance testing*

Measuring reduction of an injected substance in the blood over time can be used to estimate renal clearance and therefore GFR. Most common exogenous substances used in veterinary medicine for estimation of GFR are iohexol and creatinine. Other substances and techniques can be used, such as inulin, radiolabeled markers, and contrast-enhanced computed tomography (CT). A novel fluorescent tracer has been evaluated as a rapid, non-invasive bedside test in dogs. Ultimately, choice in method used depends on availability of the injected substance and method of measurement as well as the experience. In some cases, estimation of individual kidney GFR (vs. global GFR) is necessary, as is possible with scintigraphy or CT.

Iohexol clearance and exogenous creatinine clearance give a measure of total GFR; DTPA (a radiolabelled marker) gives estimate of total as well as individual kidney GFR. One of the main limitations with clearance methods is need for serial, precisely timed blood draws. An accurate clearance calculation requires as many as 8 post-injection blood samples over 6 hours or longer, although reasonable estimates can be obtained with limited sampling (i.e., 2 or 3 post-injection samples). Timing of these limited sample collections varies depending on the substance used. Some studies have found that calculation of plasma clearance based on a single post-injection sample is strongly correlated with 3-sample techniques, as long as an estimated volume of distribution can be determined. This is especially important in cats, where multiple collections can prove difficult. Another limitation with plasma clearance is the large amount of variability in what is considered to be “normal” in dogs and cats. In one study of 118 healthy dogs, iohexol clearance ranged from 0.95-4.25 mL/min/kg. In previously published studies in healthy dogs and cats, the range for various clearance estimates was as wide as 2.45-6.64 mL/min/kg (dogs) and 2.19-3.49 mL/min/kg (cats), although most weighted reference intervals were around 3-4 mL/min/kg (dogs) and 2.5-3.5 mL/min/kg (cats). Therefore, it is difficult to define a normal GFR in a particular animal without a baseline for that patient, and it limits ability of plasma clearance to detect early reductions in GFR. Week-to-week and month-to-month biological variability must also be considered when monitoring plasma clearance in a particular patient. Based on the week-to-week variability of iohexol clearance in a cohort of dogs with mild but stable renal disease, a subsequent measurement must increase or decrease by up to 20% in order to be 95% confident that a true change in clearance has occurred. Interestingly, despite using more measurements, each with its own inherent variability, iohexol clearance variability was similar to that for serum creatinine (sCr) in these dogs. In addition to biological considerations, analytical considerations in plasma clearance calculations are important. When using a limited sampling technique, a correction formula must be applied to correct for the initial distribution phase in order to avoid overestimation of the GFR. Correction formulas for both dogs and cats are
available when using iohexol. Normalization to body weight, surface area, or extracellular volume has been recommended, but it is not clear which normalization technique should be used in dogs and cats.

**Spot tests**

- **Urine specific gravity (USG).** USG varies from minute-to-minute and is influenced by hydration and volume status. A dilute USG on a spot sample may be normal in patients that have ingested water recently in excess of what is required for hydration. Additionally, many non-renal disorders influence USG by altering volume status and/or by inhibiting anti-diuretic hormone function in the distal renal tubule and collecting duct. Patients with persistent PU/PD may not have renal disease and other disorders should be ruled out if not azotemic (e.g. hyperadrenocorticism, hypothyroidism, hyperthyroidism, diabetes mellitus, hypercalcemia, hepatic disease, consumption of higher sodium chloride diets, administration of diuretics, supplements, or herbs with diuretic activity, etc).

- **Blood urea nitrogen (BUN).** Used as biomarker for assessment of renal function. More influenced by non-renal factors than serum creatinine – e.g. pre-renal and post-renal. Urea nitrogen is a small molecule that diffuses easily across cell membranes and into and out of tissues. With dehydration, urea nitrogen is reabsorbed in tubules and less is filtered due to decreased renal blood flow; therefore, it increases more quickly and often to a greater degree than creatinine. Urea is produced from metabolism of ammonia by hepatic urea cycle; therefore, increased intestinal protein load will result in generation of more ammonia and urea nitrogen. It should not be used as a sole biomarker for renal function and interpretation of an elevation is not possible without historical and physical examination findings and a urine specific gravity.

- **Serum creatinine (sCr).** Main endogenous marker currently used for estimating GFR. While this molecule meets most of the criteria for an ideal marker of GFR, it has several major limitations, of which the most important in veterinary medicine include breed variations in dogs and decreased production with muscle wasting, as is often the case in animals with CKD. It may overestimate renal function in cachectic, geriatric, and very young patients. Tubular secretion can increase as sCr increases, although this is thought to be minimal in veterinary species as compared with humans. These factors can make monitoring of sCr over time a less reliable indicator of declining GFR. Early in disease, limitations of sCr in an individual patient are minimal. Muscle mass is usually stable in dogs and cats with early renal disease, and tubular secretion due to an increased blood concentration is not a factor. Careful monitoring and trending of fasted serum creatinine concentrations can allow for early detection of renal disease manifested by a declining GFR. Trending sCr means that serial determinations of sCr are assessed to look for significant increases in a particular patient that are likely to reflect worsening renal function. Serum creatinine concentration lends itself to trending quite nicely because it demonstrates very little intra-individual variation in healthy, adult animals, even over several years. Small increases within the reference interval can reflect significant decreases in GFR in an individual patient, and a reference interval will not be helpful when using sCr to identify the earliest declines in GFR due to kidney disease in most patients. When comparing serial measurements of sCr with renal clearance of an exogenous substance, they correlate strongly and subtle increases in sCr can identify a decrease in GFR at a similar point in disease progression (unpublished observations). While trending of sCr can be useful in the early detection of renal disease, many patients present to a clinic with no prior bloodwork. In this case, the various factors that can influence sCr need to be considered (e.g., breed,
When trending sCr, be aware of both its biological and analytical variability. Biological variability in an individual patient is best determined by bloodwork performed during routine health checks. As long as at least 3 measurements have been obtained, one can calculate the reference change value (RCV) for a patient. This value represents that at which one can be 95% confident that an increase or decrease in the analyte has occurred. Some reference laboratories are incorporating similar statistical analyses into their reports to allow easy trending of various blood analytes. If enough previous bloodwork results are not available for a particular patient, another guide that can be used is the RCV determined in a population of dogs with mild (sCr < 2 mg/dl) but stable renal disease. Total (biological + analytical) variability of sCr determined over a 3-week period in these dogs, and the RCV was 0.2 mg/dl, meaning that an increase in sCr of 0.2 mg/dl would indicate a statistically significant increase in this marker when sCr < 2 mg/dl. In addition to biological variability, analytical variability is a factor in the assessment of sCr. Most reference laboratory instruments have excellent precision in their sCr measurement, having a coefficient of variation < 5%. The difference between sCr measurements in the same sample can be as much as 0.2 mg/dl in mildly azotemic samples using the same instrument and much higher in moderately to markedly azotemic samples or if different instruments are used. Many in-clinic instruments have minimal, if any, quality assurance programs in place to ensure optimal performance. Based on both the bias and imprecision found in instruments commonly used in veterinary laboratories, the ASCVP Quality Assurance and Laboratory Standards Committee has set their total allowable error (TE$_a$) guideline for sCr at 20%. This means that analytically variability alone could potentially account for an increase or decrease in sCr of ≥ 0.2 mg/dl. It is particularly important that serial determinations of sCr are measured on the same instrument, ideally one that is subjected to a strict quality assurance program.

**Symmetric dimethylarginine (SDMA).** This test is now provided on all biochemical panel testing through IDEXX Laboratories: https://www.idexx.com/corporate/home.html. SDMA is a small molecule that originates from hydrolysis of methylated proteins. This molecule has shown great promise as an endogenous marker of GFR as it appears to be exclusively eliminated by glomerular filtration, and significant extra-renal influences on its production and elimination have not yet been identified. It is stable in whole blood, serum, and plasma at 4°C and room temperature for up to 7 days, and it is not altered with freezing in serum or plasma. This molecule has been evaluated in several canine studies. In dogs with rapidly progressing CKD, SDMA correlated strongly with GFR estimated using iohexol clearance. Notably, when using reference intervals, SDMA identified a decrease in GFR earlier than sCr, however, when both were trended over time, no major differences in identification of declining GFR were noted. SDMA changed approximately 9 months earlier than sCr. These results support that trending of sCr is necessary for sensitive detection of decreasing GFR and that SDMA might be a useful adjunct to sCr in identification of renal disease, particularly given the tendency to classify a dog as azotemic or not based on a reference interval. SDMA is not influenced by muscle mass, but any non-renal change in GFR will impact it. For example, with dehydration there is a decrease in GFR and therefore SDMA will also be influenced. It might prove especially useful in the initial diagnosis of CKD in those patients for which sCr will not provide a reliable estimate of GFR. In cats with CKD, SDMA correlates with sCr. While preliminary data suggest that SDMA might increase beyond its reference interval before sCr in cats and that a higher SDMA:creatinine ratio might
indicate a worse prognosis. Similar to dogs, it is not influenced by lean body mass; however, non-renal factors affecting GFR will impact SDMA. SDMA changed approximately 17 months earlier than sCr.

- **Cystatin C.** Cystatin C is a cysteine protease inhibitor that is produced at a constant level in all nucleated cells. It shares many of the same properties of an ideal marker of GFR as sCr. Non-renal influences appear minimal, although administration of large doses of glucocorticoids, thyroid dysfunction, and some malignancies can increase its production. In dogs, cystatin C might be influenced by age, weight, and dietary intake, although several conflicting studies exist. The biological variation of cystatin C (inter- and intra-individual variation) is similar to creatinine in healthy dog. Its availability in veterinary medicine is still limited, and there has been no verification that the cystatin being measured is truly canine cystatin C (vs. other cystatins). In humans, the majority of studies support that cystatin C is a more sensitive and accurate marker than sCr for detecting early declines in GFR, particularly in those subpopulations in which the limitations of sCr are overtly recognized. In dogs, studies have shown cystatin C to be either comparable to or more sensitive than sCr to declines in GFR. It might therefore be a reasonable alternative to sCr in detecting decreased GFR due to renal disease. Its value over sCr and the influence of nonrenal factors on its level are still unknown in veterinary medicine. In cats, measurement of cystatin C has recently been determined using both a human-based assay and a feline-specific assay. Both of these studies demonstrated high serum and urine cystatin C concentrations in cats with CKD compared with healthy cats.

### Tests of tubular function

- **Urinalysis.** A complete urinalysis provides many indicators of renal tubular function. Urine specific gravity is determined by number of functional nephrons but tubular function determines the final concentration of urine. Urine produced by filtration is iso-osmotic to plasma and bulk reabsorption of water (in addition to solutes) occurs in the proximal convoluted tubule. The loop of Henle concentrates the urine through absorption of water but not sodium; however, in the thick ascending loop of Henle, sodium is reabsorbed but not water (active dilution). In the distal convoluted tubule, urine is again iso-osmotic to plasma but in the distal part of the distal convoluted tubule and collecting duct, final urine concentration is determined through the passive actions of anti-diuretic hormone (ADH). Glucose is freely filtered at the glomerulus and under normal situations is completely reabsorbed by proximal tubular cells. Glucose is transported from tubular fluid via transporters on the epithelial cell surface that also require sodium (sodium glucose luminal transporters; SGLT2 in the first segment and SGLT1 in the distal segment of the proximal convoluted tubule). Glucose is then transported from the intercellular space via glucose transporters (GLUT2 in the proximal segment and GLUT1 in the distal segment of the proximal convoluted tubule). Glucosuria can result from hyperglycemia (due to diabetes mellitus, excessive endogenous or exogenous glucocorticoids, or stress) or from a proximal renal tubular defect (such as primary renal glucosuria or Fanconi syndrome). Cylindruria (casts on urinary sediment) is indicative of renal tubular damage. Casts are elongated, cylindrical structures formed by mucoprotein congealing within renal tubules and may contain cells. Hyaline casts are pure protein precipitates, are transparent, and have parallel sides and rounded ends and are composed of mucoprotein. They may occur with fever, exercise, and renal disease. Epithelial cellular casts form from entrapment of sloughed tubular epithelial cells in the mucoprotein; they may be observed with renal tubular disease.
Granular casts are thought to represent degenerated epithelial cellular casts. Waxy casts have a granular appearance, and are thought to arise from degeneration of longstanding granular casts. They typically have sharp borders with broken ends. Other cellular casts include erythrocyte casts and WBC casts, and are always abnormal. Erythrocyte casts form because of renal hemorrhage. WBC casts occur because of renal inflammation, as with pyelonephritis. Fatty casts are not common, but can be observed with disorders of lipid metabolism, such as diabetes mellitus. A few hyaline or granular casts are considered normal. However, presence of cellular casts or other casts in high numbers indicates renal damage, and may be one of the earliest laboratory abnormalities noted with toxic damage to renal epithelial cells (eg, gentamicin, amphotericin B).

**Enzymuria.** Enzymatic activity, used as a marker for AKI, belong to enzymes that are found within the renal tubular cells. These enzymes are too large to be filtered through a normal glomerulus, and so in the absence of profound glomerular disease, a rise in the urinary activity of such enzymes is typically caused by acute damage to the tubules and leakage from the tubular cells. Other urinary markers of acute kidney injury such as urinary electrolyte concentrations, as well as urinary glucose and protein will be discussed elsewhere in the text. Studies have included urinary psi-glutamyl transpeptidase N-acetyl-β-glucosaminidase (NAG), gamma-glutamyl transpeptidase (GGT). Studies have been performed focusing on 24-hour urinary excretion and urine enzyme activity to creatinine ratios for GGT and NAG in experimental models as well as naturally occurring kidney disease in dogs and cats. A few things appear to be clear from reviewing the literature. Urinary enzymes, GGT, and NAG are the most commonly used and most practical enzymes to assess urinary activity. NAG is found within the proximal tubular lysosomes and GGT with the proximal tubule brush border. The activity of these enzymes is a sensitive method of detecting acute tubular kidney injury, more sensitive than changes in glomerular filtration rate, serum biochemistry (azotemia) and clinical signs. Changes in urinary enzyme concentrations, GGT and NAG, can be estimated by enzyme to creatinine ratios on spot urine samples, deeming 24-hour urine collections not absolutely necessary. Normal or baseline values vary greatly from study to study and likely from assay to assay making the determination of a healthy reference range very difficult. Thus the ideal method to use this tool today appears to be when a baseline value can be determined in an individual case and then a 2-3 fold increase in the GGT or NAG to creatinine ratio indicates an early sign of acute tubular injury. This is most feasible when a dog or cat has either very recently been exposed or will be exposed to a known renal toxin and a baseline can still be established. Examples of such cases include the use of renal toxic chemotherapeutic agents, the use of aminoglycosides, a very recent overdose of a non-steroidal anti-inflammatory drug (NSAID) or the use of an NSAID in a renal compromised patient.

**Low molecular weight proteins.** Retinol binding protein. Retinol-binding protein (RBP) is a 21-kDa low molecular weight protein (LMW) protein synthesised in the liver. Unbound RBP is filtered at the glomerulus and is almost completely reabsorbed and catabolised in the proximal tubular cells. Like other LMW proteins, RBP can be detected in the urine when there is tubulointerstitial damage impairing reabsorption. Studies in dogs suggest that RBP is a promising marker of tubular dysfunction in CKD. However, assessment of tubular function using RBP in hyperthyroid cats is problematic secondary to large inter-individual variation, and may indicate that this is not a suitable marker of feline renal tubular injury. Alpha-1 microglobulin and Beta-2 microglobulin. Alpha 1-Microglobulin, a 27-kDa anti-inflammatory protein, and beta 2-microglobulin, an 11·8-kDa protein expressed on all nucleated cells, are also LMW markers of proximal tubular dysfunction. Unlike RBP and •1-
microglobulin, a major limitation in the measurement of \( \cdot 2 \)-microglobulin is its instability in acidic urine.

- **Neutrophil gelatinase-associated lipocalin (NGAL).** Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein expressed in neutrophils as well as many epithelial cells including the renal proximal tubule, loop of Henle and collecting ducts. The urine NGAL to creatinine concentration ratio (uNGAL/c) was elevated in the early stages of X-linked hereditary nephropathy in dogs, and correlated well with serum creatinine concentration, GFR, other urine biomarkers. uNGAL/c was elevated in healthy puppies possible due to urinary contamination with preputial leukocytes. In dogs with AKI, urinary NGAL concentrations increased earlier than a detectable elevation of creatinine outside the reference interval. NGAL is elevated secondary to tubular damage, but is not kidney-specific.

- **Fanconi test.** Urinary hyperexcretion of amino acids, phosphate, glucose, HCO\(_3\)-, Ca\(^{2+}\), K\(^{+}\), and other ions, and proteins of molecular weights under 50,000 daltons, in conjunction with RTA and ADH-resistant polyuria, defines the complex tubulopathy termed Fanconi syndrome. There are inherited and acquired forms of Fanconi syndrome. The pathogenesis of the syndrome regardless of its cause involves one of two basic mechanisms. The first is that renal tubular membranes become leaky, allowing less efficient reabsorption of solutes. The second hypothesis suggests that the intracellular metabolism of renal tubule cells fail to produce sufficient energy to support transport. Any substance that could be “toxic” and alter renal tubular metabolism, such as heavy metals (e.g., lead, copper, mercury, organomercurials, Lysol, maleic acid) and drugs (e.g., gentamicin, cephalosporins, outdated tetracycline, cisplatin, salicylate) could impair transport processes. Fanconi syndrome may also occur with malignancies (e.g., multiple myeloma), monoclonal gammopathies, hyperparathyroidism, K\(^{+}\) depletion, amyloidosis, nephrotic syndrome, vitamin D deficiency, interstitial nephritis associated with antitubular basement membrane antibodies, or as a complication of renal transplantation. Dogs with Fanconi syndrome have abnormal fractional reabsorption of many solutes. Reabsorption of glucose, phosphate, and amino acids is abnormal in all affected dogs. Aminoaciduria is generalized in most dogs, but occasionally is limited to cystinuria with minor defects in reabsorption of methionine, glycine, and some dibasic amino acids. Many dogs also have variably severe reabsorptive defects for HCO\(_3\)-, Na\(^{+}\), K\(^{+}\), and uric acid. The disease usually is identifiable in adult dogs when there is glucosuria and low urine specific gravity with a normal blood glucose concentration. Proteinuria usually is mild. Metabolic acidosis is variable in severity and hyperchloremic in nature, as expected with decreased proximal tubular reabsorption of HCO\(_3\). Hypokalemia can occur with longstanding disease, and may contribute to muscular weakness in some dogs. Azotemia and hyperphosphatemia are observed in dogs with advanced disease and renal failure. Renal clearance studies to identify reabsorptive defects for electrolytes and amino acids are necessary to differentiate Fanconi syndrome from primary renal glucosuria. A Fanconi test is available through PennGen: [http://research.vet.upenn.edu](http://research.vet.upenn.edu) and a 3-5 ml sample of urine is submitted. The Orthopedic Foundation for Animals [http://www.offa.org/index.html](http://www.offa.org/index.html) provides genetic testing on saliva using an FTA card for dogs with naturally occurring Fanconi Syndrome.

- **Fractional excretion (FE).** Fractional excretion (FE) is a measure of the percentage of a substance filtered by the kidney which is excreted in the urine. It is calculated as follows:
  - \( \text{FE (\%)} = \frac{\text{GFR} \times (P_x - U_x \times V)}{V} \), where \( P_x \) = plasma concentration of substance 'x', \( U_x \) = urine concentration of substance 'x', and \( V \) = urine volume.
  - Because GFR is determined by the filtration of creatinine, fractional clearance formula rearranges:
- \( \text{FE (\%)} = 100 \times \left( \frac{U_x \times V}{P_x \times \left( U_{CR} \times V \right) / P_{CR}} \right) \)
- \( \text{FE (\%)} = 100 \times \left( \frac{U_x \times P_{CR}}{U_{CR} \times P_x} \right) \)

Where: where \( P_x \) = plasma concentration of substance 'x', \( P_{CR} \) = plasma concentration of creatinine; \( U_x \) = urine concentration of substance 'x', \( U_{CR} \) = urine concentration of creatinine, and \( V \) = urine volume

Fractional clearance can be used to evaluate renal tubular function (electrolytes, amino acids, etc) as well as azotemia using FE of sodium where < 1%: is pre-renal, 1-2% indeterminant, and >3% acute tubular necrosis.

Additional testing related to renal disease

- **Indirect arterial blood pressure**
  - Doppler: this utilizes ultrasonographic waves that are transmitted by a piezoelectric crystal and is reflected back to the crystal and then converted to audible sound. It utilizes the Doppler shift effect. Blood in an artery is moving while surrounding tissue is not. It is very good for systolic blood pressure, but is not very accurate for measuring diastolic and mean arterial pressure. A cuff is placed over the artery proximal to placement of the piezoelectric crystal. The crystal is placed on a shaved area over the artery. The cuff is inflated above systolic blood pressure so no flow of blood occurs in the artery. The cuff is slowly released until blood flow is re-established, which is the systolic blood pressure. A sphygmomanometer (gauge) is used to give a numeric value to the systolic pressure.
  - Oscillometric: this utilizes the principle of movement (oscillations) and the intensity of vascular wall vibration (movement) from the pressure. It can determine systolic, diastolic, and mean arterial pressure. Although useful, it is less accurate than Doppler. A cuff attached to the oscillometric blood pressure instrument is placed over an artery. No clipping is necessary. Pressure in the cuff is increased until it exceeds systolic blood pressure and no flow of blood occurs in the artery. The instrument slowly releases pressure from the cuff and detects vascular wall vibrations as blood flow is re-established.
    - The first vibration = systolic
    - The most intense vibration = mean
    - The point where vibrations level off = diastolic
  - Indirect arterial blood pressure is determined over the palmar metacarpal, cranial tibial, or coccygeal arteries. It is important to perform when patient is not stressed; therefore, having the owner hold, use minimal restraint, perform away from people and other patients, and perform prior to sample collection and physical examination. Systemic arterial hypertension may occur in 65-75% of dogs and cats with CKD.

- **Proteinuria.** Finding of proteinuria should be interpreted in light of other findings on urinalysis. Always examine urine sediment to rule-out inflammation, infection, or hemorrhage, which is associated with proteinuria. Proteinuria with an inactive sediment may indicate glomerular disease. The protein pad on urine dipstick is a semi-quantitative measure using a colorimetric method. The amino groups of proteins binds to an indicator in filter paper producing a color change that is graded subjectively to a standard. It is most sensitive to presence of albumin but reacts with other proteins in the range of 30-3,000 mg/dl. Recent studies suggest this analytical pad is not very good with many false positives and false negatives. False positives occur with alkaluria, disinfectants and cleaners containing quaternary ammonia compounds, prolonged contact of the pad with urine, and
any pigment that absorbs onto the pad resulting in a color change. False negatives may occur with very dilute or acidic urine and presence of some abnormal proteins such as Bence Jones proteins that do not cross-react with the pad. Although some laboratories use sulfasalicylic acid to verify the dipstick pad findings, it has been found to be inaccurate in dogs and cats. Evaluate the dipstick in light of the urine sediment examination (active versus inactive). As little as 10% whole blood (volume/volume) can result in a positive dipstick reaction. Inflammation and infection can result in substantial proteinuria due to exudation of inflammatory proteins.

Recently, an “early diagnosis of renal disease” (ERD) test has become available, which determines urinary albumin in the range of 1-30 mg/dl. It is highly specific and sensitive because it is an antigen capture ELISA. It may be useful in detecting early renal disease associated with glomerular proteinuria; however, 20-35% of dogs presenting for veterinary care may test positive and not have progressive disease.

If proteinuria is present with a “clean” sediment and a bacterial urinary tract infection has been ruled-out, then the degree of proteinuria should be verified and quantitated. Although a 24-hour urine sample can be collected for determination of 24-hour urinary protein excretion, a urine protein-creatinine ratio (UPC) is an easier test and correlates with 24-hour urinary protein excretion. A spot urine sample can be collected by any method (as long as hemorrhage is not induced although it takes substantial blood – more than microscopic hematuria – to affect UPC). Normal UPC in dogs and cats is < 0.2:1.0; up to 0.4:1.0 in cats and 0.5:1.0 in dogs is suspect, and greater than these ratios is abnormal. Treatment depends on whether azotemia is present or not.

- **Infectious disease.**
  - **Bacterial infection.** Aerobic bacteriological culture is the gold standard for diagnosis of bacterial urinary tract infection. Urine should be collected by cystocentesis and submitted by changing the needle to a sterile needle and injecting into a sterile container. Commercially available urine culture collection tubes containing preservative, which may or may not be combined with refrigeration, may be used to preserve specimens for up to 72 hours before processing occurs.
    - Point-of-care bacteriological testing. Several in-house of point-of-care bacteriological testing are available.
      - In-house culture plates. Blood agar and MacConkey’s agar plates may be inoculated and incubated for 24-48 hours. A calibrated bacteriologic loop or a microliter mechanical pipette that delivers exactly 0.01 or 0.001 mL of urine to the culture plates should be used to estimate cfu/mL, and urine should be streaked over the plates by conventional methods. Blood agar supports the growth of most aerobic bacterial uropathogens, and MacConkey’s agar provides morphologic information that aids in the identification of bacteria and prevents “swarming” of *Proteus* spp. Plates are incubated or placed under an incandescent light. If bacterial growth is noted within 48 hours, the plates may be submitted for identification and determination of antimicrobial sensitivities.
      - Flexicult. An agar plate with one compartment for quantitative analysis using a chromogenic substrate allowing for bacterial identification and 5 antibiotic impregnated compartments: ampicillin, amoxicillin plus clavulanate, cephalothin, enrofloxacin, and trimethoprim-sulfamethoxazole. Accurately excludes urinary tract infection but less reliable for diagnosing infection, especially with Gram-positive cocci. Most of the antimicrobial susceptibilities had only fair concordance with standard microbiological culture technique.
EZ-PZ. A rapid catalase based urine-screening test. Screens for bacteriuria, hematuria, pyuria and the presence of other somatic cells. A positive result indicates that urine requires further diagnostic evaluation.

Indicator RX. This is a 24 hour test that detects the presence of bacteria in canine or feline urine samples. Identifies bacteria as one of the primary gram-negative uropathogens (i.e., *Escherichia coli*, *Klebsiella*, *Enterobacter* spp., and *Proteus* spp.) that are responsible for feline and canine urinary tract infections (UTI). Predicts the antibiotic resistance pattern for the UTI-related gram-negative bacteria found in canine and feline urine samples. Device is composed of 5 test wells, labeled “BAC” (bacteria), “GM(-)” (Gram negative), “FQ” (fluoroquinolone), “AMO” (amoxicillin), “CEP” (cephalosporins – first generation) and 2 control wells labeled “POS” (positive) and “NEG” (negative).

Uricult Vet. A UTI screening by providing a semi-quantitative colony count along with a presumptive identification of many common uropathogens. Product consists of a two sided paddle containing selective and non-selective media that fits securely into a screw cap plastic vial to maintain sterility. One side contains C.L.E.D. agar that changes color in the presence of various organisms including E. coli, Proteus, *Pseudomonas*, Enterobacter, and others. The opposite side contains EMB (Eosin Methylene Blue) agar, a selective medium that will support the growth of most Gram negative organisms while providing additional information regarding the suspected pathogen.

**Leptospirosis**

Leptospirosis is a zoonotic bacterial disease with a worldwide distribution, and is an emerging infectious disease in humans\(^1\) and in dogs. Disease in dogs is caused primarily by *Leptospira interrogans* and *Leptospira kirschneri*. The most common serovars thought to infect dogs before the introduction of leptospirosis vaccines 30 years ago were Icterohaemorrhagiae and Canicola. Since the introduction of bivalent Icterohaemorrhagiae and Canicola vaccines, more widespread involvement of additional serovars has been suspected, including Grippotyphosa, Pomona, Bratislava, and Autumnalis.

**Diagnosis:**

- Microagglutination Test (MAT). Use of antibody testing for diagnosis of leptospirosis generally is based on the MAT, which involves reacting serial dilutions of patient sera with an array of live leptospiral serovars, and assessment of organism agglutination by darkfield microscopy. The highest serum dilution causing agglutination of 50% of the leptospires in the reaction is reported. The MAT is widely available and inexpensive, and there is a large body of data regarding its use; as such, it is the current diagnostic test of choice for canine leptospirosis in patients with consistent clinical signs. Test interpretation is somewhat subjective and requires considerable expertise, and serovar identity must be verified regularly to ensure accurate results. Considerable variation in results has been noted among laboratories performing the MAT for diagnosis of canine leptospirosis, possibly as a result of variable quality control and standardization. There is a lack of consensus over what titer should be used as a cut-off for a negative result. The MAT is a serogroup- rather than a serovar-specific test, because antibodies to serovars within the same serogroup cross-react extensively. In the 1\(^{st}\) week of illness, dogs frequently have negative MAT results, and consequently acute and convalescent phase antibody testing is recommended. Traditionally, convalescent titers for acute infectious disease diagnosis are performed 2–4 weeks after the acute titer, although seroconversion can occur as early as 3–5 days after dogs are brought to a veterinarian. Wait 7–14 days between successive titers to demonstrate seroconversion.
A 4-fold change in titer supports recent infection, although an increase in titer may be blunted by antimicrobial therapy. Titers resulting from previous vaccination, exposure, or chronic infection generally change more slowly or not at all. Titers can persist for at least 1 year after natural infection, and in 1 study, generally declined by 4 months after vaccination. Postvaccinal titers may persist for longer and be maintained at high levels if ongoing exposure to field strains occurs. Thus, although single positive titers can increase suspicion for the disease, even when high (≥800), they do not confirm a diagnosis of leptospirosis. This is especially important in dogs with a history of vaccination, because although postvaccinal titers tend to be low, high titers (≥1,600) have the potential to persist after vaccination, and cross-reactivity to nonvaccinal serogroups can occur. In 1 study, the sensitivity of a single MAT titer ≥800 for diagnosis was 22–67%, depending on the laboratory used, and the specificity was 69–100%.

ELISA. The lipoprotein LipL32 is the most abundant outer membrane protein found in pathogenic species of *Leptospira*. An enzyme-linked immunosorbent assay (ELISA) for the detection of LipL32 antibodies in the dogs is now available. Provides a qualitative positive or negative antibody result. Similar to MAT testing, some currently vaccinated dogs may have detectable antibodies on the assay. Duration of vaccinal antibody reactivity may vary depending upon the dog and frequency of vaccination.

Polymerase Chain Reaction (PCR). Detects pathogenic nucleic acid and has potential utility early in course of untreated infection when antibody assays are frequently negative and antimicrobials have not yet been administered. Can confirm active infection in animals with positive antibody test results that have a history of vaccination with leptospiral vaccines, because previous vaccination should not yield false positive results by these methods. They may detect infection in dogs with chronic renal or hepatic disease. In the first 10 days of infection, organism numbers are highest in blood, and thus blood is the sample of choice during the first week of illness. After that time, organisms are present in highest concentration in urine. When the time of infection is unknown, simultaneous testing of blood and urine may increase diagnostic sensitivity. Recent antimicrobial treatment can result in false negative test results for both culture and PCR, although multiple doses of antimicrobials may be required before PCR becomes negative, because PCR detects both viable and nonviable organisms. Although PCR assays have been designed to detect only pathogenic leptospiral serovars, currently available assays do not differentiate between serovars or serogroups. Negative results do not rule out leptospirosis, because they may occur when organism numbers in a sample are low, or other factors, such as PCR inhibitors, are present. Currently, there is limited information regarding the validity of PCR assays for detection of pathogenic leptospires infecting dogs, as well as their sensitivity, specificity, and positive predictive value, and so positive and negative test results should always be interpreted in conjunction with other diagnostic methods such as acute and convalescent phase antibody testing.