

Paws claws and udder things

Issue 2, Spring 2009



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Dr. Allan Kessell has kindly expanded on a fact sheet from Gribbles Veterinary on Urinalysis. His article will answer many of the questions some of you have asked on this subject. Also you will see we have appointed three new pathologists over the last few months. They are experts in their field, which will ensure we provide you with the best service you deserve.

If you have any suggestions on items you would like to see in the newsletter, or if you would like to comment on some of our subject matter, please feel free to contact us.

Uses and Limitations of Urinalysis

by Dr. Allan Kessell, Specialist Veterinary Pathologist, Gribbles VETLAB, SA

Analysis of urine is an important part of the examination of the urinary system in health and disease. A fact sheet from Gribbles Veterinary Pathology entitled "Urinalysis" has previously been circulated that contains much useful information, and here some additional limitations inherent in the methodology are highlighted.

Urinalysis is best performed on a fresh, room temperature sample of urine (or a cool preserved sample that has been let come back to room temperature). Cystocentesis samples do not contain contaminants from the lower urinary tract, although iatrogenic haemorrhage may occur. A full analysis consists of 4 separate procedures :

- 1) **Gross examination of the urine** – the colour may help in detection of haematuria, haemoglobinuria or myoglobinuria. Sediment exam (see below) will allow separation of haematuria (significant red blood cells – RBCs - in sediment exam) from haemoglobinuria (red supernatant on spinning sample). Myoglobinuria is best detected by measurement of serum creatinine kinase (CK), which will be high when there is significant enough myonecrosis to cause myoglobinuria. Strong colour development due to haematuria/haemoglobinuria/myoglobinuria will interfere with colour changes used for dipstick biochemistries, often invalidating them.
- 2) **Measurement of urine specific gravity (USG)** – the value is dependent on the amount of water consumed, the composition of the diet and the ability of the kidney to excrete or conserve water. USG is one measure of renal functional capacity, although generally speaking at least two-thirds of the nephrons must be damaged before it is measurably affected. Animals with normal renal function may have dilute, non-dilute or concentrated urine, dependant on the amount of water consumed. This measure is very useful in helping differentiate the causes of polyuria and the significance of any measured azotaemia. Also, importantly, this measure is required to assess the significance of the level of some chemical analytes e.g. a 1-2+ protein in concentrated urine is not unusual, but in dilute urine implies proteinuria in the absence of an active sediment (that is, in the absence of haematuria or pyuria).

Limitations : assess USG in light of azotemia and water consumption. Some small molecular weight substances will cause mild false elevation of USG when in excess e.g. glucose, ketones. It is also best to use a refractometer calibrated for dogs/large animals and for cats to estimate USG. Human refractometers will mildly overestimate the concentration of feline urine.

Uses and Limitations of Urinalysis

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- 3) **Biochemical analysis** – typically allows semi-quantitative measures of urinary pH, glucose, ketones, bilirubin, urobilinogen, blood, and protein. It is important that the test be performed with test strips that are in date, and have been stored in a bottle with dessicants. The significance of results should be viewed in light of the USG.

- **pH** : generally reflects the acid/base status of the animal, and any animal may have an acidic or alkaline urine, although carnivores generally have acid urine whilst herbivores produce alkaline urine. However alkaline urine may indicate some infections are more likely (urease producing bacteria like Staphylococcus and Proteus spp.), and when >8.5 may also result in a false positive protein reading and cause the disintegration of proteinaecous structures like RBCs and WBCs (thus interfering with accurate sediment examination). Also an incorrect pH reading may be seen if run over of urine from the protein reagent patch is allowed to occur (contains an acid buffer).
- **Glucose** : most strips are based on glucose oxidase (eg. Multistix) which is specific for glucose, and help in detecting transient and persistent glucosuria. Glucose will appear in urine when serum levels exceed 9-10 mmol/L in dogs and 13-17 mmol/L in cats, both of which may occur transiently under stress in these species, although this is more common in cats (and cows and horses). Glucose may appear in the urine in the absence of hyperglycaemia in some renal tubular disorders eg. Fanconi syndrome (as recently seen with dogs eating some brands of processed food). Ascorbic acid (Vit C) may give rise to a low false positive.
- **Ketones** : often indicate a shift from oxidisation of glucose to fat as an energy source, and can be seen with a variety of conditions, including diabetes mellitus, glucose sparing states (hypercortisolaemia), starvation or high fat diets. When testing for ketones a fresh sample is recommended, as excessive exposure to light may give rise to a false positive result; a trace false positive can also occur in highly concentrated urine with a low pH. Test strips do not detect beta-hydroxybutyric acid, and are much more sensitive in detecting acetic acid than acetone (all of these are ketone bodies). Thus one may have ketonuria and not be able to detect it, although this is not common. Agents that contain free sulphhydryl groups may cause false positive results eg. d-penicillamine, cystine.
- **Bilirubin** : bilirubinuria may precede clinical jaundice, and thus may be an early indicator of a hepatopathy. However, normal male dogs may have 1-2+ bilirubinuria with no bilirubinaemia, as the canine kidney may produce bilirubin under normal conditions. Hyperbilirubinaemia without bilirubinuria suggests a false negative for bilirubinuria. This can occur if bilirubin is degraded to biliverdin (not detected on dipstick) with exposure of sample to UV light (30min). In cats, in contrast, even small amounts of bilirubin are abnormal, and prehepatic/hemolytic, hepatic and post hepatic causes should be investigated.
- **Urobiliniogen** : is not considered a reliable test to detect prehepatic, hepatic or post hepatic disorders in animals, but it is mentioned here as it is included on most test strips designed for human use.
- **Blood**: the test system used will give a positive result for whole RBCs, haemoglobin and myoglobin, as it detects haeme containing porphyrins. Haematuria may be confirmed by sediment examination (presence of whole RBCs), keeping in mind that dilute urine (USG<1.008) or highly alkaline urine may cause haemolysis in the sample. Haemolysis or myoglobinuria is detected in red/brown urine when the supernatant from a spun sample retains its colour; myoglobin will be associated with high CK associated with muscle damage.

Urinalysis is best performed on a fresh, room temperature sample of urine (or a cool preserved sample that has been let come back to room temperature).

Uses and Limitations of Urinalysis

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- **Protein** : protein is contained in plasma, cells and various bodily fluids. The presence of protein in the urine may be due to prerenal, renal or post renal disorders. Pre renal disorders may be transient and result in mild proteinuria e.g. fever, or represent conditions of high serum protein overload e.g. some neoplastic B cell disorders. Renal disorders may be further divided into glomerular and post glomerular (tubular, interstitial, renal pelvis). Glomerular disorders are associated with the highest amounts of urinary protein (in relation to USG), as glomerular damage will allow high molecular weight proteins such as albumin to pass and result in tubular protein overload and significant proteinuria. Chronic tubular protein overload may eventually lead to direct tubular damage. Inflammatory, ulcerative and neoplastic conditions in the kidney or lower urinary tract (or reproductive tract if free catch collection is used) may result in renal or post renal protein loss respectively. The significance of proteinuria must be assessed in light of possible prerenal causes (usually mild proteinuria); renal causes - possible glomerulopathies, interstitial/tubular/pelvic disease, and post renal causes. Tubular damage results in casts in urine, nephritis/pyelonephritis/cystitis in WBCs and possibly RBCs in urine, and neoplasia may result in RBC/WBCs or neoplastic cells in urine. A urinary protein:creatinine (UPC) will allow a more quantitative measure of urinary protein loss and assessment of the degree of glomerular damage, once inflammatory and neoplastic causes of protein loss are eliminated i.e. a UPC can only be interpreted in light of an inactive urinary sediment in which there are no significant numbers of RBCs/WBCs.

“whilst urinalysis is a very useful procedure , results are best interpreted in light of history, clinical signs, and an understanding of the limitations inherent in the test methodology.”

Note that contamination with some quaternary ammonium compounds (some disinfectants) and highly alkaline urines may result in false positive protein results. A 1-2+ reading may be seen in highly concentrated urines in normal animals.

- 4) **Sediment examination** : the laboratory often receives requests for urine cytology with or without urinalysis, and yet a standard laboratory urinalysis will **always** include a cytologic examination of the unstained sediment of concentrated urine. Requesting both cytology and urinalysis will result in additional charges for stained cytology, and stained cytology often yields poor results (see below). Unless neoplasia is suspected and specific **stained** cytology of the urine is required to try and detect neoplastic cells, standard sediment examination performed as part of the laboratory urinalysis will allow accurate detection and semi-quantitation of RBCs, WBCs, casts, crystals, normal (and sometimes abnormal) epithelial cells, and fungi. If neoplasia is suspected **fresh** urine is required, as the cellular elements degenerate quickly in unpreserved urine, and a detailed examination of such stained specimens is often expensive and unrewarding. This is problematic, as distance from the laboratory, courier times etc. may result in aged urine arriving at the laboratory. Timing urine collection with courier runs, keeping the urine cool, and preserving some in EDTA (purple top tube) may help if stained cytology is required i.e. if neoplasia is suspected.



Sediment examination is rarely performed at the veterinary clinic, as it is time consuming, and requires considerable skill and experience in accurately identifying unstained cells/bacteria/crystal type etc. The urine must be spun down at low speeds, and 90% of the supernatant discarded, with the remaining button resuspended and examined under a microscope with a dropped condenser (allowing better visualisation of unstained elements). Specific cut-offs are well documented in the literature for acceptable levels of RBCs (<5 per 400 x field), WBCs (< 5 per 400x field), casts (<2 per 100x field) etc., and allow one to comment on pyuria, haematuria, tubular damage etc. The presence of crystals must be interpreted in light of other findings, as their presence is affected by age and temperature of the urine, pH, diet etc. Urolithiasis may be seen in the absence of crystalluria, whilst crystalluria is often seen in the absence of any urolithiasis.

Summary : whilst urinalysis is a very useful procedure , results are best interpreted in light of history, clinical signs, and an understanding of the limitations inherent in the test methodology.

Our Team of Experienced & Dedicated Staff



Dr Daren Hanshaw

Our Latest Recruits

We are delighted to announce that three new pathologists have joined the team - Daren Hanshaw, Geoff Orbell and Liz McInnes.

Daren qualified as a veterinary surgeon in 1987 at Murdoch University. He spent some years both in Australia and the UK in practice before moving to the US to pursue a career as a veterinary anatomical pathologist. He subsequently worked for Finn Pathologists in the UK and then returned to Adelaide to join Gribbles at our Glenside laboratory.

Geoff graduated from Massey University in 2001. He spent two years in a large progressive production animal practice in New Zealand. Following this he spent time in mixed practices in the UK. Geoff obtained the American Veterinary Board exams in anatomic pathology in 2008, after a teaching residency at Washington State University. Geoff can be found at our Clayton laboratory in Victoria.

Liz is based at our Glenside laboratory in SA. She has 10 years experience in toxicology pathology and a special interest in immunology, cross reactivity studies and pathology artefacts. Liz qualified as a veterinary surgeon in 1988 from University of Pretoria in South Africa. She then completed a PhD, before taking on a residency at the University of Cambridge. She was awarded the Membership of the Royal College of Pathologists in 1997 and the Fellowship in 2005.

We wish to welcome Daren, Geoff and Liz to the Gribbles Veterinary team and we wish them every success in their new roles.



Dr Geoff Orbell

Investment in our Laboratories

Liver Fluke Testing in South Australia

Gribbles Glenside laboratory began performing faecal egg sedimentation testing for liver fluke in October 2008. This test is performed in accordance with the method approved by Agriculture WA for animals entering Western Australia (which is liver fluke free). This compulsory test for all livestock movements into Western Australia can be performed within a few days of stock moving and prevents unnecessary delays once animals are on the move. Reports for stock moving to WA are sent to the processing station in Kalgoorlie to keep the process moving smoothly.

This test can also be performed for private vet clients where cases of liver fluke are suspected.

A minimum 10g faecal sample (grab sampled) is required for horses and a minimum 4g sample for cattle and sheep.



Dr Liz McInnes

Tina Sizer-Taylor
Laboratory Manger

Gribbles VETLAB, Glenside, SA

Check out our New Profiles and Tests

In response to feedback we have added some new profiles to our existing range. These include:

- **Annual Health Profile (canine/feline)**
- **Geriatric Profile (canine/feline)**
- **Sick Dog/Cat profile**

A new test of interest to our Equine practices is the **Serum Amyloid A test (SAA)**. SAA is a major acute phase protein in horses. Acute phase proteins increase with inflammation and other acute phase proteins include fibrinogen and haptoglobin.

SAA is well suited not only for diagnosing the presence of inflammation, but also for real-time monitoring of inflammatory changes in disease states and responses to treatment and surgery.

We have restructured the Blood Trace Mineral Profile for Production Animals into three formats:

- **Full Trace Mineral Profile**
This includes: Cu x 10, GPX x 10, Vit B12 x 10
Sample required: Whole blood & serum or plasma x 10 animals
Container: LH x 10
- **Near Full Trace Mineral Profile**
This includes: Cu x 10, GPX x 2 (pooled into 2 groups of 5 animals), Vit B12 x 10
Sample required: Whole blood & serum or plasma x 10 animals
Container: LH x 10
- **Shortened Trace Mineral Profile**
This includes testing of 2 groups of 5 animals: Cu x 2 (pooled), GPX x 2 (pooled), Vit B12 x 2 (pooled)
Sample required: Whole blood & serum or plasma x 10 animals
Container: LH x 10

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ACT tubes available

The MAX-ACT™ and the C-ACT™ activated clotting tubes are now available

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C-ACT™ tubes 2mLs blood
Cost: \$3.25+GST/tube*

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Cost:\$4.95+GST/tube*



For further details contact your local CSM or our Help Desk on 1300 307 190 or check out our website.

*Note: * Minimum order 5 tubes/pack*