Marshfield Labs offers two tests to aid in the diagnosis of pheochromocytoma.
1. Metanephrine Fractionation, Urine, Canine (VMETRND)
2. Catecholamine Fractionation, Urine (VCATRND)

NOTE: Order VMETRND first; if test results are inconclusive or normal, and a high suspicion of pheochromocytoma exists, then order VCATRND as a second test.

BACKGROUND

Pheochromocytomas are catecholamine-producing neuroendocrine neoplasms of the adrenal medulla and may be functional (hormone-producing) or nonfunctional. Functional tumors produce catecholamines (epinephrine, norepinephrine, and dopamine).

Clinical signs may be vague but usually result from secretion of excessive amounts of catecholamines or their metabolites (metanephrine, normetanephrine). Because hormone secretion is episodic and unpredictable, the clinical presentation varies greatly. Clinical signs of hypertension including weakness, episodic collapse, tachypnea, panting, tachycardia, pacing, polyuria/polydipsia have been reported. With acute, episodic release of catecholamines, you may see acute myocardial necrosis, arrhythmia, congestive heart failure, ventricular fibrillation, pulmonary edema, and cerebral hemorrhage. It is unclear if hypertension results from catecholamine release by the pheochromocytoma or from other concurrent diseases. Pheochromocytomas tend to be locally invasive and vasoinvasive, often infiltrating the caudal vena cava; thus, signs attributable to the space-occupying or invasive nature of the tumor, including ascites and hind limb edema, may occur.

DIAGNOSIS

There has been no widely used test for the diagnosis of pheochromocytoma in animals.

In humans, total excretion of urinary catecholamines and their metabolites (metanephrine, normetanephrine) is the most widely used tool for diagnosing pheochromocytomas. A diagnosis of
pheochromocytoma is based on increased excretion of these compounds. Marshfield Labs now offers testing for urinary excretion of catecholamines and their metabolites (metanephrine, normetanephrine) to aid in the diagnosis of functional pheochromocytomas in animals.

The diagnosis of pheochromocytoma relies on combined interpretation of clinical history, physical examination findings, diagnostic imaging of an adrenal mass, and laboratory testing. A definitive diagnosis of pheochromocytoma however, relies on histopathology of adrenal tissue. Affected patients show no consistent abnormalities on CBC, biochemical profiles, or urinalysis.

Diagnosis of functional pheochromocytomas is further complicated by a number of factors including the variety of hormones produced by these tumors, the stability of these hormones, and their episodic release. Patients with hypertension of any cause may have increased concentration of plasma and urinary catecholamines and catecholamine metabolites. Some drug therapies and non-adrenal tumors may also increase urine and plasma levels of catecholamines and their metabolites. To complicate matters, occasionally pheochromocytomas in humans are reported to produce ACTH and result in hyperadrenocorticism, therefore hyperadrenocorticism is one of the most important differential diagnoses for pheochromocytomas. A urine cortisol:creatinine ratio can be evaluated to rule out hyperadrenocorticism. Consequently, increased urinary catecholamine or metanephrine/normetanephrine concentration is not specific for a diagnosis of pheochromocytoma.

Test methodology variation also complicates result interpretation. Several publications evaluating normal dogs, dogs with hyperadrenocorticism, and dogs with pheochromocytomas utilize high pressure liquid chromatography (HPLC) assays for urinary hormone assessment. Marshfield Labs utilizes HPLC combined with mass spectroscopy analysis (HPLC MS/MS) for detection of catecholamines, metanephrine, and normetanephrine. The HPLC MS/MS methodology is much more specific than HPLC alone. It is difficult to determine if the overlap in results between healthy dogs, dogs with hyperadrenocorticism, and dogs with pheochromocytomas reflects the relative low specificity of the HPLC method. One of the studies using HPLC methods suggested an interpretive threshold of 4 times the upper limit of normal for urinary metanephrine/normetanephrine to creatinine ratio is needed to increase the specificity of the assays in the diagnosis of pheochromocytoma. Similar threshold limits are employed for interpretation of urinary catecholamine and metabolite values in humans. Assessment of these hormones by the more specific HPLC MS/MS technology may yield more specific results.

Both VMETRND and VCATRND approximate 24 hour urinary hormone excretion. The urinary assays for these hormones are reportedly more specific but less sensitive than plasma assays in studies performed in humans. Metanephrine/normetanephrine assays may be more sensitive than epinephrine and norepinephrine because these metabolites are more stable than epinephrine and norepinephrine; hence, why performing the VMETRND test first is recommended.

False positives can result from exercise, excitement, radiographic contrast agents, and certain medications. Conversely, reduced renal function and intermittent secretion by the tumor may result in lower values.
TEST INFORMATION

Test Code:
- **VMETRND** (recommended as the primary assay to screen for pheochromocytoma)
- **VCATRND**

Specimen (Patient and Control):
For each, submit 3-5 mL urine samples in a sterile container to which 0.15 grams or a few grains of sulfamic acid are added. Acid is added to stabilize the sample and is supplied by Marshfield Labs upon request.

Limited studies show nonacidified, refrigerated samples appear stable for approximately 48 hours. Acidified samples are preferred for best results.

Control Samples:
- **VMETRND**: Submit urine control samples for species other than dogs.
- **VCATRND**: Submit urine control samples for all species when ordering.

Reference intervals for urinary **VMETRND** dogs:
- Normetanephrine/Creatinine ratio = 28 – 380 ug/g Creatinine
- Metanephrine/Creatinine ratio = 18 – 359 ug/g Creatinine

Reference intervals for urinary **VCATRND** dogs:
- Norepinephrine/Creatinine = 0.001 - 0.037 ug/mg Creatinine
- Epinephrine/Creatinine = 0.000 - 0.019 ug/mg Creatinine
- DOPA/Creatinine = 0.000 - 0.031 ug/mg Creatinine

Interpretation:
A four-fold increase in **VMETRND** or **VCATRND** patient results above the reference interval or control specimen, and in association with patient clinical history, physical exam findings, and diagnostic imaging, supports a diagnosis of pheochromocytoma.

Because pheochromocytomas secrete hormones episodically, serial assessment of urinary catecholamines and metabolites may be needed to rule out the presence of this tumor.

REFERENCES

Every few weeks we receive a cytology specimen that has been exposed to formalin rendering the specimen unreadable. This typically occurs when you have simultaneously submitted a formalin specimen for histopathology and a specimen for cytology.

Exposure of cytology specimens to formalin causes prominent morphological artifact, altering staining properties, causing a blue-green haze to the cells, thus markedly diminishing morphologic detail for the pathologist. Ultimately this leads to the necessity of resampling and resubmission at additional cost/time to your client.

Please avoid exposure of any cytology specimens to formalin. Cytology samples should be submitted in a separate container from formalin-fixed samples or double-bagged. Formalin vapors leak through capped or screw-top lids and affect staining quality as illustrated below. (Figures 1 and 2.)

**Figure 1.**
Formalin Artifact: Canine Liver Cytology
(50X oil, Diff-Quik® stain)
Note the blue-green haze and diminished cellular detail.

**Figure 2.**
Normal Canine Liver Cytology
(50X oil, Diff-Quik® stain)
No formalin artifact.

For more Cytology Pearls, use the link below to access a previous article, “How to Get the Most Out of Your Cytologic Sample: Collection and Processing Techniques” by our pathologist Jennifer Brazzell, DVM, Diplomate ACVP, in the Reference Point, Spring 2010 issue.

https://www.marshfieldlabs.org/proxy/RefPointSpring10.1.pdf

(Continued on page 5)
1. To coverslip or not……

Often times we find ourselves looking at a slide through what appears to be a haze. This is most often due to a high dry (40X) objective lens that is dirty/caked with oil, and simply cleaning the lens will improve cell detail. However, another simple trick that will help you to visualize cells more clearly is to place a single drop of oil on the slide and then coverslip it, then examine the coverslipped slide with a 40X lens objective. This will diminish the air/lens refraction interface that is normally present and improve cellular detail. This is helpful with both blood and cytology smears.

No coverslip (hazy).  
Coverslip on top (clarity).

2. Is It REAL or ARTIFACT?

A.
B.

ANSWERS

A. Stain precipitate often mimics bacteria, including cocci and *Mycoplasma* species. Stain precipitate is typically purple and irregular, and often is in a different plane of focus than the cells. Bacteria stain blue (with a Wright’s stain), are more uniform, and are in the same plane of focus as the cells. Note that RBCs are slightly fuzzy because they are in a different plane of focus than the stain precipitate.

B. Bacterial cocci phagocytized by neutrophils. Note the band forms and toxic nature of the neutrophils (increased cytoplasmic basophilia, Dohle bodies). The bacteria are in the same plane of focus as the cells they are in.

THREE ONLINE WAYS TO ACCESS MARSHFIELD VETERINARY SERVICES

*Shelley Van Proosdy, MT(ASCP), Sr. Account Executive & Betsy Aird, DVM, PhD, DACVP*

- Marshfield offers online access to ordering lab tests, submitting pathology specimens, and obtaining results in ways to fit all practices.
- In today’s digital world, more and more practices are going “paperlight” and Marshfield Labs can help clients accomplish this through our online access options.
- In general, online lab requests reduce the amount of paperwork, the amount of redundancy, and the number of transcription errors that occur with paper orders.
- Being connected also offers clients the ability to access results anytime from anywhere that has an internet connection.

1. WEB PORTAL

The web portal is an exclusive service for our clients, providing secure, confidential access to our services via the Internet. There is no cost for this service and it’s easy to get signed up.

(Continued on page 7)
Through the Web Portal, clients can:

- Order tests online - resulting in less paperwork and reduced delays due to handwritten request forms
- Access patient test results
- Check the status of a test
- Request additional testing
- Order supplies
- Download test results (See sample screens below.)

Also available on the Portal:

- Test Directory: Complete test and sample submission information
- Billing: Look at past invoices or the month in progress and pay bill online
- Trending reports: Create test volume reports or generate graphs showing testing trends by patient or date range
2. DIRECT INTERFACE

Marshfield Labs offers a direct interface to ImproMed Infinity and Triple Crown practice management software (PMS) systems.

Through the Direct Interface, users can:

• Place orders within ImproMed
• Capture all charges - resulting in no lost revenue from lab testing
• Receive results back into the patient medical record automatically

3. INTEGRATION VIA VETLABS

Marshfield Labs offers a seamless connection to all other major PMS through the use of VetLabs Integration™.

VetLabs Integration™ offers:

• EASY INSTALLATION
  VetLabs Integration™ is installed on your practice server and can also be installed on any number of practice workstations. VetLabs runs side by side with the practice management system and is as easy to access as a module within the PMS.

• NO DOUBLE ENTRY
  When lab work is added within the PMS, the requisition is automatically started. Your clinic's invoice codes are mapped to our test codes.

• REQUISITION TRACKING AND CAPTURED CHARGES
  When the practice clicks the create button, the request for testing is sent to Marshfield Labs. The samples are sent to the lab and status updates are displayed within VetLabs.

• RESULTS DELIVERED
  Once the results are available, VetLabs will retrieve them and notify you that new results are available.

• SECURE AND LIGHTWEIGHT
  VetLabs Integration™ securely provides the right information at the right time through an efficient app residing on your toolbar.

If you are interested in one of the online access options, please contact Customer Support at 800-222-5835. Your Marshfield Labs Account Executive will be happy to help you get set up.