ANTECH DIAGNOSTICS

News, Advice and Research from ANTECH Diagnostics

ANTECH INSIGHTS

August/September 2012
Focus: Renal Disease Diagnostics

Role of Quantitative MA Testing in Everyday Practice
Interview with Brenda Harai, DVM, MS, DACVIM
ANTECH Diagnostics Internal Medicine Consultant

Urinalysis Revisited:
Reminders for Optimal UA & Urine Culture Submissions

Why Send Out your UAs?
The Answer Lies in the Sediment

Client Alert! ProZinc Insulin Back-Ordered

Introduction

In this issue, we highlight important pieces of the renal disease diagnostic puzzle. While many veterinary hospitals routinely rely on **urine dipstick** followed by confirmatory **SSA** testing to screen for proteinuria in both ill and healthy patients, peer-reviewed research published in the past two years has demonstrated the poor overall diagnostic utility of these tests and the importance of pairing them with more sensitive & specific quantitative tests for proteinuria. In particular, these tests have poor sensitivity for detecting **microalbuminuria (MA),** a key biomarker for the presence of underlying disease with secondary glomerular damage, and the progression of chronic kidney disease (CKD) in both dogs and cats. We hope our discussion of MA testing prompts discussion in your practice regarding its appropriate role in early disease detection and kidney disease screening, monitoring and management.

Role of Quantitative MA Testing in Everyday Practice

Interview with Brenda Harai, DVM, MS, DACVIM
ANTECH Internal Medicine Consultant

Q: *Can you remind us what microalbuminuria indicates?*

A: **Albumin** is the main component of urinary protein and is what we are most interested in for assessment of renal proteinuria, a condition that can be due to glomerular disease, tubulointerstitial disease, or inflammatory or infiltrative disorders of the kidneys.

Microalbuminuria is defined as amounts of albumin greater than normal (>2.5 mg/dL) in the patient's urine, but often below the limit of detection of conventional dipstick urine protein screening (<= 30 mg/dL) and what has been considered "normal" using a urine protein:creatinine (UPC) ratio. Microalbuminuria has been associated with a variety of non-renal infectious, inflammatory, neoplastic, metabolic, and cardiovascular disease. It can also be seen with glucocorticoid excess & hypertension. It is an **early marker of renal disease**, both tubular and glomerular in origin. Urine albumin concentrations greater than 30 mg/dL are referred to as **overt albuminuria**. The urine protein/creatinine ratio (UP/C) is an important methodology for quantifying proteinuria in this higher range.

Q: *How does microalbuminuria (MA) testing compare to the urine dipstick, SSA test, and UP/C ratio?*

A: Generally speaking, microalbuminuria testing such as the quantitative MA tests offered by ANTECH (species-specific turbidometric immunoassays) is much more sensitive and specific for albuminuria than the urine dipstick, SSA test or UP/C ratio, per the recent JAVMA study published by S. Lyon *et al*[1] (partial summary of results below).

The study authors concluded that these three methods have poor overall diagnostic performance – **particularly with feline urine samples**. Three specific conclusions of this 2010 study are important to consider:

- When the urine dipstick and SSA screening test results are ***in the trace to 1+ range, adding on the urine microalbumin test is recommended to confirm the presence of albuminuria.***
- Based on the poor diagnostic performance for feline urine samples, ***detection of albumin in feline patient urine should always be performed with a higher-quality assay,*** such as the species-specific quant MA test.
- The ***UP/C should not be used as a routine screening test*** for the detection of albumin in clinically normal dogs or cats given its insensitivity for detecting low level albuminuria and consequently the unacceptable number of false negative results.

When the 2004 ACVIM Consensus Statement on the Assessment and Management of Proteinuria in Dogs and Cats[2] was published, the data from this study and two other important JAVMA studies published in 2010 were not yet available. Perhaps if the statement was published today, a stronger role for quantitative MA testing might be suggested. [Note that ACVIM Consensus Statements are available at no charge on the ACVIM.org web site.]

SUMMARY OF RESULTS FOR URINE DIPSTICK, SSA AND UP/C TESTS FOR DETECTION OF ALBUMINURIA[1]

Canine Results

Test	Sensitivity	Specialty
Urine Dipstick	81.2%	47.8%
SSA	73.3%	63.9%
UP/C ≥ 0.5	28.7%	99.7%

Feline Results

Test	Sensitivity	Specialty
Urine Dipstick	90.1%	11.0%
SSA	58.0%	25.4%
UP/C ≥ 0.4	2.04% (33% with 0.2 cut-off)	99.2%

Q: *When is it most important to serially follow or monitor a dog's or cat's urine albumin concentration?*

A: Per the 2004 Consensus Statement, it is critical to monitor patients with apparently progressive chronic kidney disease (CKD) as well as patients with stable, subclinical CKD. Adequate monitoring (at least every 6 months) of patients with subclinical CKD should detect deteriorating renal function in a timely fashion – which is critical for appropriate intervention to attempt to slow progression of renal disease.

It's important to note that in animals with CKD causing renal failure, proteinuria may actually diminish as the patient reaches end-stage kidney failure, because fewer and fewer functional nephrons will remain through which protein loss can occur. In this scenario, it's critical to interpret MA or UP/C results in light of serum creatinine levels.

Q: *What are the indications for MA testing?*

A: I recommend the MA test for the following five scenarios:

APPLICATION:	WHEN/CLINICAL SITUATION:
Follow-up testing	All cats and dogs with **positive dipstick or SSA test results** to rule out false positives (typically trace or 1+) and to quantify the degree of albuminuria.
Monitoring	**Patients with previously elevated MA tests** to determine persistence and progression of albuminuria.
Screening	Breeds prone to **familial renal disease** (e.g., Soft Coated Wheaten Terriers) for early detection of proteinuria.
	Patients with chronic **diseases associated with proteinuric renal disease** for early detection of proteinuria (a few examples include: dirofilariasis, chronic inflammatory diseases such as periodontal disease, otitis, pyoderma, IBD, neoplasia, Lyme disease, ehrlichiosis, FeLV, FIV, and Cushing's syndrome).
	Clinically normal dogs and cats for early detection of proteinuria as part of **routine early detection plans** (along with CBC and blood chemistry), particularly older patients who have a higher prevalence of microalbuminuria.

Q: *Will blood or an active sediment affect the MA test result?*

A: Microscopic hematuria (i.e., from cystocentesis) does not affect the MA test. However, urinary tract inflammation (as evidenced by pyuria and/or bacteriuria) and gross hematuria may affect results.

Q: *Finally, what if the MA value is abnormal?*

A: First, be sure the urine sediment is inactive and there is no urinary tract infection. Assess the animal for conditions associated with physiologic proteinuria. Repeat the MA test in 7-10 days to ensure the microalbuminuria is not transient.

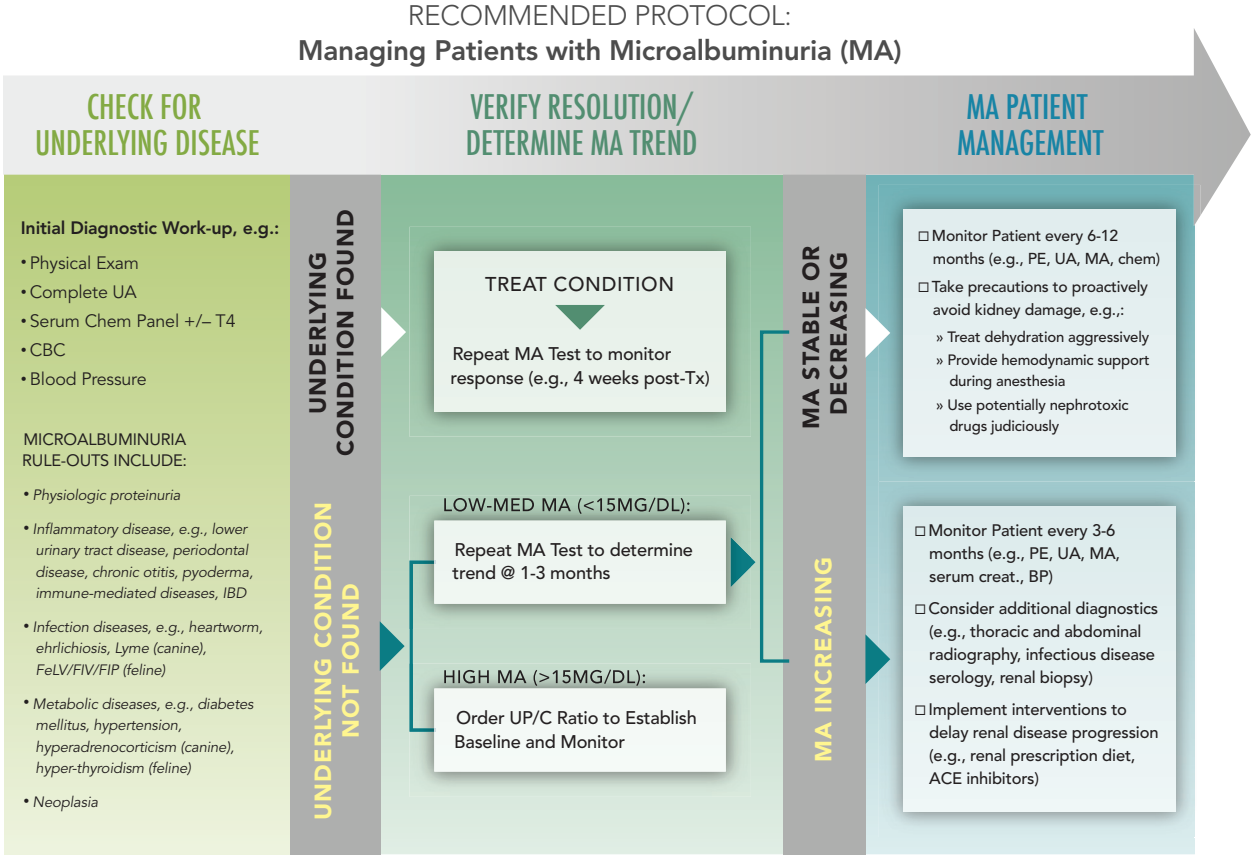
If the result is persistent, look for underlying diseases (such as pyoderma/otitis, periodontal disease, dirofilariasis, hypertension, chronic GI disease, hyperthyroidism) with a history, physical exam, serum chemistries, CBC, blood pressure, T4 (cats), and heartworm antigen test. If an underlying disease is found, **recheck the MA in 1-3 months** after treatment and resolution.

If no underlying disease is found on initial diagnostics, the next step depends on the magnitude of the MA. If the MA is in the **mid-to-upper half of the pathologic range (e.g., >15 mg/dL), a UP/C ratio should be run** to determine if treatment is warranted at this time (see ACVIM Consensus Statement). Consider further diagnostics such as infectious disease testing (e.g., for Lyme disease, ehrlichiosis, leptospirosis, FeLV, or FIV), thoracic and abdominal imaging.

If the MA is in the **lower half of the pathologic range (e.g., < 15 mg/dL)**, consider **repeating the MA in 1-3 months.** If the degree of MA is stable or decreasing, ongoing monitoring (e.g., PE, UA, MA test, and serum creatinine) is recommended every 6-12 months. If the magnitude of the MA is increasing over time, consider additional diagnostic procedures (e.g., thoracic/abdominal imaging, infectious disease serology, blood pressure, CBC/blood chem, UP/C ratio) to further evaluate the patient.

In closing, clients should always feel free to contact the ANTECH Consult line to discuss applications and limitations of MA testing.

RECOMMENDED PROTOCOL:
Managing Patients with Microalbuminuria (MA)



References:

1 Lyon, S, et al. JAVMA 236:874-879 (Comparison of urine dipstick, sulfosalicylic acid, urine protein-to-creatinine ratio, and species-specific ELISA methods for detection of albumin in urine samples of cats and dogs.)
2 Lees, G. E., Brown, S. A., Elliott, J., Grauer, G. F. and Vaden, S. L. (2005), Assessment and Management of Proteinuria in Dogs and Cats: 2004 ACVIM Forum Consensus Statement (Small Animal). Journal of Veterinary Internal Medicine, 19: 377–385.
3 Whittemore, J, et al. JAVMA 230: 1165-1169 (Association of microalbuminuria and the urine albumin-to-creatinine ratio with systemic disease in cats.)
4 Whittemore, J, et al. JAVMA 229: 958-963 (Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs.)
5 Grauer, G. Vet Clin Small Anim 37:283-295 (Measurement, Interpretation, and Implications of Proteinuria and Albuminuria)
6 Grauer, G. Vetoquinols Academia Proceedings 2007 (Early diagnosis of Chronic Kidney Disease: Focus on proteinuria, albuminuria, and microalbuminuria)

Urinalysis Revisited:
Reminders for Optimal UA & Urine Culture Submissions

CBC & Chem without UA? Time to reconsider. If a chemistry profile reveals azotemia, how do you know whether it's renal or pre-renal azotemia without having a UA collected at that same time? According to the 2004 ACVIM Consensus Statement on Canine and Feline Proteinuria, **urinalysis should be a component of any evaluation of ill patients that includes a CBC and serum chemistry panel.** Similarly, the consensus statement recommends that urine testing that detects proteinuria should be a component of any routine health evaluation of apparently healthy dogs and cats that includes a CBC and serum chemistry test.

Urine collection. The plain ("no additive") RTT is the preferred collection tube for urinalysis and urine culture. A Copan swab is not preferred for urine culture, as it precludes quantitation of results.

UA SAMPLE INSTRUCTIONS

CORRECT

Submit at least 3ml urine in a plain red-top Vacutainer tube.

Always submit two (2) urine tubes to allow for a urine culture add-on based on the UA findings. Place 3 mls in tube for UA and 1ml in tube for culture.

AVOID

RT Vacutainers marked as "serum" and large urine containers with screw caps.

Urine culture. Because of the relative insensitivity of routing urinalysis for detecting bacteriuria, occult cases of urinary tract infections (UTI) can be overlooked if urine is not cultured. Urine culture is the most sensitive and specific test for diagnosing UTI.

UA CULTURE INSTRUCTIONS

CORRECT

Submit 1ml urine in a plain red-top tube or in one of the plain urine tubes with snap-on translucent caps provided for ANTECH EAST clients.

AVOID

Do not submit culture swabs of urine (Copan or any other type of swab)

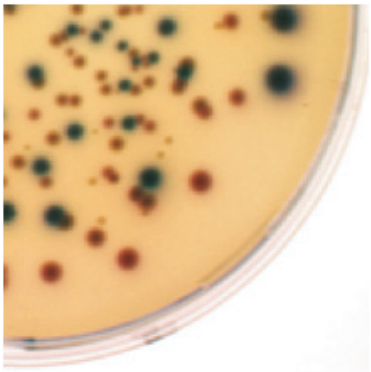
Urine for culture is best collected by **cystocentesis** and transported with a cold pack to prevent bacterial overgrowth. The site should be cleaned with alcohol or disinfectant to minimize the risk of contamination by cutaneous flora. Make sure the animal is off antibiotic therapy for at least 48-72 hours prior to cystocentesis to prevent suppression of bacterial growth.

The ANTECH Urine Culture (Test Code: M130) **includes MIC antibiotic sensitivity testing** for positive results.

MIC testing guides antibiotic selection not only by determining whether the tested bacteria are **sensitive or resistant** to any given antibiotic, but importantly by determining the relative efficacies of antibiotics to which the isolate is sensitive (that is, which antibiotics are most likely to result in treatment success). The "susceptibility breakpoint" used to interpret MIC results is the concentration of antibiotic slightly greater than that required to kill sensitive strains of bacteria. Thus, the further the MIC for a particular antibiotic is below its susceptibility breakpoint, the more effective that antibiotic is likely to be against that particular bacterium.

Choosing an antibiotic for UTI. Two key points to remember in using the urine MIC panel:

- All other things being equal (cost, safety, ease of use, compliance), choose the antibiotic with the lower MIC relative to its susceptibility breakpoint;
- The closer the MIC to its resistant breakpoint, the higher the dosage of antibiotic needed, and vice versa.

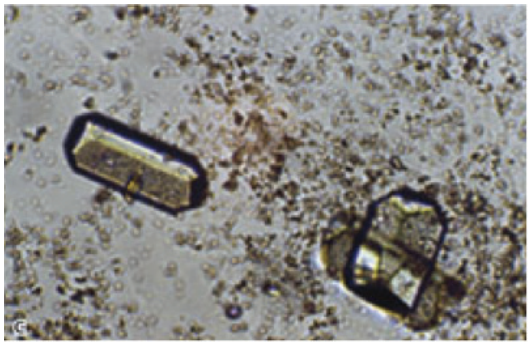


Urine colony counts. In addition to urine culture, urine colony counts or quantification of urine cultures can be performed to determine the number of bacterial colonies per mL of urine. This may be helpful, when urine is not collected by cystocentesis, in assessing whether the bacteria isolated and identified on culture are possible contaminants or significant pathogens.

Results of urine colony counts are reported as the number of colonies (or the range) per mL of urine along with the identification of the organism and antimicrobial sensitivity. For samples collected via catheterization, bacterial counts ≥ 10,000/ml in males and ≥ 100,000/ml in females are typically considered significant. Positive culture results in samples collected by midstream voiding are not considered reliable and re-culturing to verify the presence of UTI is recommended. To avoid the whole condundrum, use cystocentesis whenever possible to procure urine for culture. The highest reported value for bacterial counts is >100,000/ml.

Note on Urine sample stability. Routine transport time does not significantly influence urinalysis and has no effect on urine culture results if the sample has been collected appropriately and placed in the correct type of sterile container.

Why Send Out Your UAs?
The Answer Lies in the Sediment



A study conducted by ANTECH to compare the accuracy of UAs performed by veterinary hospital technicians with comparable reference lab testing revealed some important deficiencies in the in-house UAs, most notably in **sediment evaluation** errors.

Study design. Fifteen animal hospitals (2-4 DVMs each) participated in the 2009 study, which replicated an external quality control/proficiency check. Test samples were sent to each hospital to be run and have results returned for evaluation versus baseline results determined by ANTECH. Six (6) urinalysis specimens were sent to each hospital (90 total urine samples) over a 5-day period. Additional aliquots of the same samples were sent to other ANTECH lab locations with similar handling for comparison to baseline results and to confirm sample stability. Results were interpreted based on variance from the baseline results, determined by running the samples in duplicate at ANTECH's Irvine lab and again at another ANTECH lab location to confirm they matched the original results (following transport with the same protocol as samples shipped to the 15 participating hospitals). In general, a variance of +/-20% of the bas eline value was considered acceptable.

Results. The following variances between in-house urinalysis and reference lab results were most common:

URINE CHEMICAL ANALYSIS:	
False positive bilirubin results	Many errors were attributed to manual interpretation of color changes
False positive protein measurements	Attributed to known limit of urine strip proteins and misreading of dipstick pad color changes

URINE MICROSCOPY:	
Bacterial cocci false positives	25% of samples with negative sediments were reported by in-house labs to have bacterial cocci because of misinterpreting sediment debris as cocci (especially a problem in cats with concentrated urine)
Inconsistent crystal identification	Struvite crystals identified as Oxalate in 10 of 90 specimens

Among the discrepancies, ANTECH found the **high false positive rate for bacterial cocci** to be the greatest concern, given that it **commonly leads to unnecessary UTI treatment.**

Urine sediment analysis at ANTECH. The discrepancies between in-house and reference lab UA testing are attributable to the significant differences in technician focus and training that ANTECH's scale allows. ANTECH reference lab technicians are trained specifically to read urine sediment, with rigorous online training and testing before the technicians begin to do UAs for clients. The technicians are backed up by board-certified pathologists who review unusual abnormal findings (e.g., clusters of or abnormal transitional epithelial cells, fungal elements) or anything with which the technicians are not familiar. Cocci bacteria and low numbers of bacilli found by the technicians are also verified by staining air-dried sediment slides for verification.

Finally, in addition to an array of daily quality assurance steps and controls for all lab equipment involved in UAs, ANTECH participates in quarterly proficiency testing, which involves blinded studies that evaluate all aspects of ANTECH UA testing.

Related Antech Test Codes:

CODE	TEST NAME	TURNAROUND
T760	Urinalysis (with sediment evaluation)	Daily
M130	Urine MIC Culture	2-4 Days
T830C	Canine Urine Microalbumin	Daily
T835C	Canine Urine Microalbumin Reflex	Daily
T830F	Feline Urine Microalbumin	Daily
T835F	Feline Urine Microalbumin Reflex	Daily
T775	Urine Protein/Creatinine Ratio	Daily

Related Screening Profiles: See Profile Section of ANTECH Test Directory

ProZinc Insulin Back-Ordered!

Important Alert for Feline Diabetic Patients

Boehringer Ingelheim Vetmedica has announced that there will be a temporary shortage of their ProZinc human recombinant insulin product. This is due to a key ingredient source supply problem, and not in any way related to a safety or efficacy issue. It is anticipated that supplies will return to normal by early autumn.

In the interim, it is recommended that newly diagnosed diabetic cats be started on an alternate insulin product. We recommend that newly diagnosed diabetic cats be started on glargine insulin (Lantus®). If a change in insulin type is needed for a feline diabetic patient currently regulated with ProZinc insulin, glargine or NPH insulin is suggested. Use of compounded PZI insulin is not recommended.