



ANTECH
D I A G N O S T I C S

News

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FRUCTOSAMINE AND GLYCOSYLATED HEMOGLOBIN

Fructosamines are serum proteins (albumin and others) that have undergone nonenzymatic, insulin-independent glycosylation. Serum fructosamine concentration is proportional to the blood glucose concentration over the lifespan of the glycated protein being measured. The lifespan of albumin in dogs (and presumably in cats) is 1-2 weeks. Thus, the serum fructosamine concentration should reflect the mean blood glucose concentration over the preceding 7-10 days.

Likewise, glycosylated hemoglobin (Hb) is produced from an irreversible, nonenzymatic, insulin-independent binding of glucose to Hb. In this way, it is an indirect assessment of the mean blood glucose for the preceding several weeks, depending on the half-life of the red blood cells (RBC). Since RBC lifespan in dogs is 120 days, the glycosylated Hb reflects the mean blood glucose concentration over the last 8-12 weeks, while in cats it reflects the mean blood glucose concentration over the preceding 5-6 weeks (feline RBC half-life of 66-78 days).

Both fructosamine and glycosylated Hb concentrations are generally lower in normal animals than diabetic animals. Furthermore, diabetics with good glycemic control (as assessed by mean blood glucose concentrations and clinical signs) have lower fructosamine and glycosylated Hb levels than animals with poor glycemic control; and both values tend to decrease as glycemic control improves. In addition, while cats with stress hyperglycemia have significantly higher blood glucose concentrations than normal cats, there is no difference between the two groups when evaluating fructosamine or glycosylated Hb levels.

Test Limitations

Although these 2 tests tend to reflect blood glucose levels, there is some overlap between diabetic and non-diabetic animals as well as between animals with good and poor glycemic control. Even cats with good glycemic control tend to have fructosamine levels higher than the normal range. Thus, it is always necessary to evaluate these tests in light of other clinical signs.

Changes in serum albumin levels will alter fructosamine levels. Low concentration of albumin or plasma proteins will result in lower than expected fructosamine concentrations while a low rate of protein turnover can increase serum fructosamine levels. In a similar manner, anemia will markedly decrease the glycosylated Hb concentration.

A recent study compared both tests and concluded that fructosamine concentration reflected glycemic control better than glycosylated Hb and more accurately reflected changes in glycemic status. In addition, measuring glycosylated Hb requires specialized techniques so that most laboratories send the samples out for measurement which increases both the cost and turn around time.

Although an elevated fructosamine or glycosylated Hb level may indicate less than ideal glycemic control, these values alone cannot tell the clinician how to alter the insulin treatment in order to improve control. For example, a dog that is receiving too little insulin will have a high fructosamine level, but so will a dog receiving too much insulin. The overdose can lead to the Somogyi effect and the subsequent hyperglycemia will lead to an elevated fructosamine concentration.

FRUCTOSAMINE AND GLYCOSYLATED HEMOGLOBIN (CONT'D.)

Clinical Use

Glycosylated Hb is probably most useful as an indicator of glycemic status in animals with well-controlled diabetes. Either test can be used to differentiate stress hyperglycemia from diabetes mellitus *when evaluated together with results of fasting blood glucose, urine glucose and clinical signs.*

Preliminary studies indicate that sequential evaluation of fructosamine concentration before and after use of insulin may be helpful in confirming changes in glycemic control.

Fructosamine concentration also can help differentiate cats with poor glycemic control from cats that have become fractious or stressed during determination of blood glucose curves. The best way to monitor an extremely fractious cat may be to anesthetize the animal and insert a jugular or other long catheter into the medial femoral vein, with enough extension tubing to permit blood sampling without stressing the patient or clinic staff. This also allows for a good physical examination, blood and urine collection and radiographs, if necessary.

Determining fructosamine concentrations cannot obviate the need for blood glucose curves, which remain the best way to monitor diabetic animals, especially at the beginning of treatment when choosing the correct type of insulin and optimal dosing schedules. Once glycemic control is obtained, fructosamine levels can be used together with clinical signs for further monitoring. If the fructosamine level is elevated, a blood glucose curve should be performed to determine the necessary changes.

References: Plier et al, Vet Clin Pathol 27: 34-39, 1998; Elliot et al, J Am Vet Med Assoc 214: 1794-1798, 1999.

PLASMA RELAXIN ASSAY FOR CANINE PREGNANCY DIAGNOSIS

Until recently, there has been no blood or urine test that would reliably predict pregnancy in dogs. A commercial relaxin assay (ReproCHECK, Synbiotics Corp.) has recently become available as a canine pregnancy test. Relaxin hormone is produced by the ovary and placenta of pregnant bitches and becomes elevated by about mid-gestation. In contrast, relaxin concentrations are reported to be undetectable in nonpregnant and pseudopregnant dogs.

The usefulness of this assay for detecting pregnancy in dogs has been under evaluation at Antech Diagnostics. To date, we have data on 33 bitches that were bred and followed to term. Relaxin concentrations were measured at least

30 days post-breeding. Fifteen dogs had positive relaxin tests and all of them whelped live puppies. There were no false positive results. Eighteen dogs had negative relaxin tests, and 14 of these dogs had no puppies. Of the other 4 dogs, 2 each had 1 viable puppy, 1 dog had 1 stillborn puppy, and 1 had 3 puppies with 1 born dead. These were false negative relaxin test results and occurred with small litter size. An additional 2 dogs carrying small litters (1-2 pups) had positive relaxin tests just before whelping.

Conclusion: An elevated relaxin concentration is a reliable test for pregnancy in dogs. False positive results rarely occur. False negative results may occur when pregnant bitches carry small

litters, and/or one or more of the puppies are nonviable.

Relaxin should be measured at least 30 days post-breeding. Negative results should be repeated in one week. The test requires heparinized or citrated plasma, **not** EDTA or serum. The plasma should be spun down, removed from the cells and placed in another tube, and labeled as heparinized or citrated plasma.

Specimen

Requirements 0.5 mL
separated GTT or BTT plasma

Turnaround

Time 2 - 4 days

LAB TIPS

MEASURING ZINC CONCENTRATIONS IN BIRDS

Low levels of **zinc contamination** has been found in red top (RT), brown top (BT), and green top (GT) test tubes from a commonly used supplier. While the normal reference range for blood zinc concentration is 0.8-2.5 ppm, these tubes contain zinc concentrations as high as 3-6 ppm. Thus, use of these tubes could cause a normal bird to appear to have zinc toxicosis.

Microtainer™ brand tubes [either spun GTs with separator gel or spun serum separator tubes (SST)] or **royal blue top Vacutainer™ tubes** are preferred for measuring blood zinc concentrations. Please call the laboratory if you need some of these tubes.

CRYPTOCOCCAL ANTIGEN ASSAY

Antech Diagnostics has recently introduced a new ELISA based assay for cryptococcal antigen. This replaces the former method and is reported either as negative, or as positive with a quantitative endpoint titer.