

Effects of Time and Temperature on Fructosamine

Measurements of blood glucose concentrations are used to diagnose and monitor the diabetic patient. A single glucose measurement in a blood sample represents only the glucose concentration at the time of the collection. This concentration may vary and be altered by time, recent feeding, severe stress, and drug administration. These variations may lead to problems in the diagnosis or management of diabetes. So, alternatives to blood glucose measurement have been used. Glycated hemoglobin (HbA_{1c}) and Fructosamine are glycated serum proteins that reflect long-term glucose concentrations in human beings and animals. Fructosamine is a stable ketoamine compound formed when glucose reacts non-enzymatically with amino groups on proteins (mainly albumin). Fructosamine increases with persistent hyperglycemia or hyperproteinemia and decreases with persistent hypoglycemia or hypoproteinemia. The assay reflects the serum glucose concentrations for the previous 1-2 weeks, and may be used to diagnose and monitor diabetes more effectively than glucose measurements.

However, there have been problems encountered with what appears to be either fructosamine stability or a species-specific recovery problem in dogs and cats. Aliquots of canine and feline samples were sent to another reference laboratory for comparison testing by the clinical pathology laboratory at Iowa State University (ISU). The concentrations from the reference laboratory were repeatedly and significantly less than the results obtained at ISU. Since the values of the Roche controls performed at ISU were well within the expected ranges, another university veterinary laboratory was contacted to run additional samples. Samples were run at room temperature, and as frozen aliquots, and thawed aliquots.

Very little change in fructosamine recovery was seen in frozen aliquots. This might suggest that frozen samples withstand degradation for this assay. Samples at refrigerator temperature showed much less concentration recovery over time (1-2 days). Those left at room temperature for more than a few hours would appear to be unacceptable when compared to the concentration of a fresh sample. According to preliminary data, when a sample is stored at refrigerator temperature for 1-2 days, and then analyzed, the concentration could decrease from being in a range of 'poor or borderline diabetic control' into the 'good control range'. Furthermore, the same poor recovery was not seen with human-based control serum.

Jensen *et al.*, 1992 and Coppo *et al.*, 1997 did studies in dogs as part of a reference interval project and concluded that there was very little negative affect of fructosamine recovery with refrigerated or even room temperature samples over a few days. Our results are contradictory to previous findings. After reviewing several other studies involving the fructosamine assay, Roche has been the only manufacturer cited as being used in open-type chemistry systems over the past 8-10 years, but there was a change in formulation of the assay in 1999. The change of pH may affect the ability of the assay to detect protein-bound glucose as the sample ages and the pH increases, and the effect might be more pronounced at higher temperatures. Also, the human proteins may not be as sensitive to these changes as the canine and feline samples. In conclusion, a problem might exist with either fructosamine stability or species-specific recovery in dogs and cats. Our current recommendation is to submit frozen serum for fructosamine assays.

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