

Effect of storage time and temperature on total calcium concentrations in bovine blood

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Introduction

Subclinical hypocalcemia (SCH), a reduction in blood calcium concentrations without apparent clinical signs of milk fever, occurs in 25 to 50 % of early-postpartum dairy cows. Compared to their normocalcemic counterparts, these cows have an increased risk of subsequent disease developments, reduced milk production, and poorer reproductive performance. Due to the absence of an economical and accurate on-farm test, the mainstay of individual cow testing and herd-level monitoring is done through total calcium (tCa) analysis performed at regional laboratories or veterinary clinics. Due to practicality reasons, this often results refrigeration of whole blood for various lengths of time prior to centrifugation or prolonged storage of frozen plasma or serum. In order to provide bovine practitioners and researchers with the optimal method of handling samples for tCa testing, our objective was to examine the effect of storage time and temperature on tCa concentrations in bovine blood.

Materials and Methods

Blood was collected from 18 postpartum, multiparous Holstein cows on 2 farms in New York State into non-anti-coagulant (red top; serum) or lithium heparin (green top; plasma) vacutainer tubes. Serum or plasma was harvested within 2 h of collection and analyzed immediately (0 h) or stored for 7 days, or 1, 3, 6 and 12 months at -80°C. Samples were also stored as whole blood at 4°C and serum and plasma were harvested after 6, 24, 48 and 72 h, or 7 and 14 d of storage. The tCa was measured at the New York State Animal Health Diagnostic Center (Cornell University, Ithaca, NY) using a Roche Modular P Chemistry Analyzer based on o-cresolphthalein complexone method. Repeated measures models were developed in SAS to evaluate the effect of storage temperature (i.e., 4°C and -80°C) using PROC MIXED with

unstructured covariance for plasma and serum tCa concentrations over time. Correction for multiple comparisons of tCa concentrations for stored samples versus 0 h were done using Dunnett's method. For pairwise comparisons between all samples, Tukey's method was used.

Results

The median 0 h tCa concentration for serum and plasma was 2.3 mmol/L with a minimum and maximum concentration of 1.6 and 2.7 mmol/L, respectively. For whole blood storage at 4°C, there was no difference in tCa concentration between serum or plasma ($P = 0.4$), however there was an effect of time ($P = 0.001$). This effect of time was not due to differences observed when comparing 0 h samples to any length of storage (all $P > 0.1$) but due to pairwise differences observed between various stored samples (24 h versus 14 d and 7 d versus 14 d). As our research question focused on whether tCa concentrations from stored samples would differ from 0 h samples, these differences observed between various stored samples are therefore inconsequential and likely due to analytical variation. There was no difference observed in tCa concentrations between plasma or serum following storage of serum or plasma at -80°C ($P = 0.6$) as well as no effect of time ($P > 0.1$).

Significance

Our findings show that whole blood samples may be stored up to 2 weeks at 4°C in red or green top tubes with no changes in tCa concentrations beyond that expected for analytical variation. Storage of serum or plasma at -80°C has no effect on tCa concentrations up to 12 months.