Stability of Homocysteine in EDTA gel tubes

Lyn Boscato and Graham RD Jones
Department of Chemical Pathology, St Vincent’s Hospital, Sydney

**Introduction and Aim**

- Homocysteine concentration is known to increase in EDTA whole blood samples by up to 40% in three hours unless the sample is maintained at 4 degrees.
- Sample cooling is often difficult to manage in the remote collection center environment.
- We evaluate the use of EDTA tubes with gel separator as a way of avoiding the requirement for cooling blood for homocysteine measurement prior to processing in the central lab.

Homocysteine was measured using the Abbott AxSYM Homocysteine assay.

**Results**

- There was no difference in the basal homocysteine concentration obtained with the standard EDTA tube and the gel separator tube (Figure 1).
- There was less than 5% average increase in homocysteine in the gel tubes under any of the storage conditions up to 24 hours, and less than 12% at 48 hours (Figure 2).
- Re-spinning the tube after 48 hours resulted in a 40% increase in the homocysteine level but no increase after 9 hours (study 2).
- There was an 80% increase in the homocysteine level of the most mixed standard EDTA tube after 9 hours compared for a 2% increase for the tube that was undisturbed (Figure 3).

**Study 1: EDTA vs EDTA Gel**

**AIM**

- To investigate the effect of blood collection in an EDTA gel tube on homocysteine measurement

**METHODS**

- 15 paired standard EDTA (EDTA) and EDTA tubes with gel separator BD PPT #362795 (EDTA Gel) were collected from healthy volunteers. Lithium Heparin gel tubes were also collected from 3 subjects.
- Tubes were placed on ice immediately, and then centrifuged at 4 degrees within 30 min of collection. Aliquots were stored frozen at -25 degrees until assay.

**Figure 1.** Regression analysis of the homocysteine level (umol/L) obtained with EDTA gel tube compared to standard EDTA tube at zero time.

**Study 2: Stability in EDTA Gel Tube**

**AIM**

- To study the effect of different storage conditions on the homocysteine level obtained in EDTA gel tubes

**METHODS**

- Blood was collected from 5 healthy volunteers into three EDTA tubes with gel separator.
- Following centrifugation the tubes of blood were stored and aliquots of plasma taken at various times up to 48 hours and frozen.
- The EDTA gel separator tubes were stored at room temperature (RT), 30 degrees (30) or on a sample rocker at room temperature (rocked).
- Tubes were also re-spun at the end of the study and two separate tubes were re-spun 9 hours after collection.

**Figure 2.** Average homocysteine level (% of level at zero time) obtained with EDTA gel tubes stored under different conditions for times as shown. 100 +/- 10% and 5% levels are shown.

**Study 3: Stability in Standard EDTA Tube**

**AIM**

- To study the effect of mixing on the homocysteine level obtained in standard EDTA tubes

**METHODS**

- Blood was collected into three standard EDTA tubes
- Tubes were stored at room temperature and aliquots of plasma taken at various times up to 9 hours. Any centrifugation steps were at 4°C. Aliquots were stored frozen.
- Tube 1 (EDTA Span) was initially centrifuged then stored stationary and not re-spun
- Tube 2 (EDTA) was mixed prior to each time point and then re-centrifuged
- Tube 3 (EDTA mixed) was mixed throughout the incubation period and before re-spinning at each time.

**Figure 3.** Homocysteine level (% of level at zero time) obtained with standard EDTA tubes stored under different conditions for times as shown.

AACB Annual Scientific Meeting, Sydney, 2005