



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.

Study 1: EDTA vs EDTA Gel

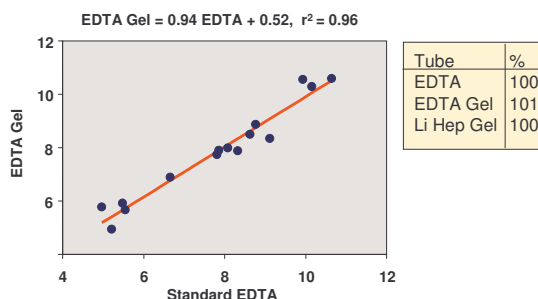


Figure 1. Regression analysis of the homocysteine level (umol/L) obtained with EDTA gel tube compared to standard EDTA tube at zero time.

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

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 2: Stability in EDTA Gel Tube

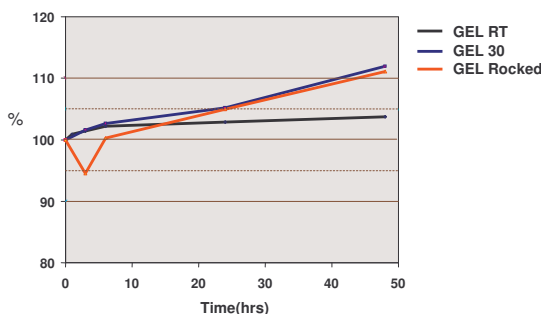


Figure 2. Average homocysteine level (% of level at zero time) obtained with EDTA gel tubes stored under different conditions for times as shown. 100 +/- 10% and 5% levels are shown.

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.

Conclusions

- ❖ The increase in homocysteine observed with standard EDTA tubes at room temperature is dependant in part on the degree of mixing of plasma with the red blood cells.
- ❖ The homocysteine level in the EDTA gel separator tube is not altered by an increase in temperature, repetitive tube movement or increased time; conditions that could be encountered during transit from the remote collection centre to the main laboratory.
- ❖ The data from this study indicates that the EDTA gel separator tube is suitable for homocysteine and will reduce the need for cooling the blood.

Study 3: Stability in Standard EDTA Tube

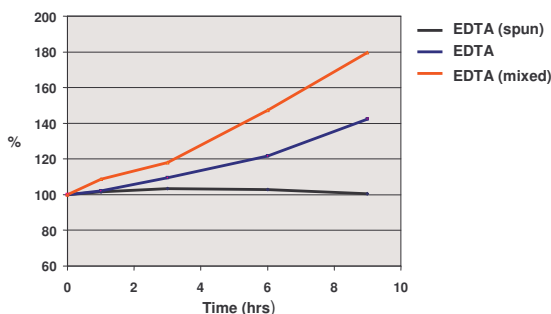


Figure 3. Homocysteine level (% of level at zero time) obtained with standard EDTA tubes stored under different conditions for times as shown.

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 Spun) 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.