

Effects of Blood Collection Tubes on Determination Vitamin-A by HPLC

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Abstract

Background: VACUETTE® Blood Collection Tubes are used for the collection of venous blood. VACUETTE® tubes are used to collect, transport, store and process blood for testing serum, plasma or whole blood in the clinical laboratory for professional use.

Objective: Evaluate the effect of blood specimen collection methods of the VACUETTE Serum Sep Clot Activator tube (gel tube), in determination vitamin A by HPLC.

Methods: Blood specimen was divided into three parts, Three tubes, for Part I, No Additive tubes were drawn, for Part 2 Serum Sep Clot Activator tube (gel tube), and for Part 3 EDTA-coated plastic tubes. The tubes were gently mixed using ten complete inversions immediately following blood collection and centrifuged after venipuncture for 15 min and the supernatants were stored at -70°C until analysis. Vitamin A Testing was performed on healthy blood donors; the testing was performed by HPLC.

Results: Our study showed that, There was no difference in the results were obtained with the serum (No Additive tube) and plasma tube in concentration of retinol 67.7 ug/dl and 73.8 ug/dl, but the results was 9.2 ug/dl obtained with the VACUETTE® Serum Sep Clot Activator tubes (gel tube).

Conclusions: Tubes are drawn in a specific order to avoid the possibility of erroneous test results caused by carryover of an additive. If Serum tube-with or without clot activator or gel, it should be drawn as the first tube. We recommended that only serum and EDTA-plasma samples be used for Vitamin A determination by HPLC and cannot be carried out with all gel tubes.

Keywords: No additive; Serum sep clot activator tubes gel tube; Vitamin A; HPLC

Introduction

All VACUETTE® Serum Tubes are coated with micronised silica particles which activate clotting when tubes are gently inverted.

VACUETTE® Serum Sep Tubes contain a barrier gel that is present in the bottom of the tube. The specific gravity of this material lies between the blood clot and the serum [1-3]. During centrifugation the barrier gel moves upward to the serum-clot interface, where it forms a stable barrier separating the serum from fibrin and cells. Serum may be aspirated directly from the collection tube, eliminating the need for transfer to another container. Serum Clot Activator tubes are made of plastic and are used for the collection of venous blood, tubes should be used with care when measuring therapeutic drug levels because the drug/hormone may diffuse from the serum into the gel, causing a reduction in measured drug level [4-7].

Tables 1 and 2 Serum Tubes, Yellow cap tubes, indicate type of blood collection tubes containing clot activator and separation gel [8-10]. VACUETTE® Serum tubes are used for determinations in serum

Red stopper	No additive (BD)
Red black stopper	Clot activator (Greiner)
Red yellow stopper	Clot activator/(Greiner)
Serum separator Gel (SST)	

Table 1: Tube stopper color & Type of Additive.

Anticoagulant	Inner Ring color	Cap color
Serum tube/clot activator	Black	Red
Serum Tube/gel and clot activator	Yellow	Red

Note: All tubes with White inner rings are Pedi draws only.

Table 2: Serum Tubes, Yellow cap tubes, indicate type of blood collection tubes containing clot activator and separation gel.

for routine clinical chemistry tests and hormones, serology, and TDM. TDMs were partially tested in gel tubes [11].

VACUETTE® K2EDTA Tubes are used for testing whole blood in hematology. The interior of the tube wall is coated with either K2EDTA or K3EDTA. The tube is also available with an 8% liquid EDTA solution. The EDTA binds calcium ions thus blocking the coagulation cascade.

Blood Collection Tubes

Containers containing coagulants

Gold or 'Tiger' Red/Black top: Clot activator and gel for serum separation. Red top PLASTIC tubes: Contains a clot activator and is used when serum is needed.

Containers containing anticoagulants

Purple or lavender-contains EDTA (the potassium salt, or K2EDTA). This is a strong anticoagulant and these tubes are usually used for complete blood counts (CBC) and blood films.

Plasma

Draw a sufficient amount of blood with the indicated anticoagulant

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to yield the necessary plasma volume. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. If required, separate plasma from cells by centrifugation within 20-30 minutes.

Serum

Draw a sufficient amount of blood to yield the necessary serum volume. Invert tube 5-10 times to activate clotting. Allow blood to clot at room temperature for 15 minutes.

Materials and Methods

We simultaneously collected blood samples from 10 healthy volunteers (median age, 36 years; range, 23-55 years) into plastic tubes, Blood specimen was divided into three parts, Three tubes, For Part I, No Additive tubes were drawn, For Part 2Z Serum Sep Clot Activator tube, and For Part 3 potassium EDTA-coated plastic tubes, The tubes were gently mixed using ten complete inversions immediately following blood collection and centrifuged after venipuncture for 15 min and the supernatants were stored at -70°C until analysis. Vitamin A Testing were performed on healthy blood donors; the testing was performed by HPLC.

HPLC apparatus

To assay the concentration of vitamin A, run in triplicate using HPLC system knauer with a smartline UV detector 2500, pump 1000, and manager 5000. C_{18} column [250×4.6 mm (I.d.); 5 μ m bead size]. The chromatographic separation was performed by a mixture of methanol, water (95:5 by volume) at a flow rate of 2.5 mL/min; Detection was monitored at 287 nm. Data acquisition and peak purity tests were performed with chromat V 3.1 software. The quantitative results were expressed as μ g/dl vs. control and calibrator.

Results

Our study showed that, There was no difference in the results were obtained with the serum (No Additive tube) and plasma tube (Figure 1 and Figure 2) in concentration of retinol 67.7 μ g/dl, 73.8 μ g/dl, but the results was 9.2 μ g/dl obtained with the VACUETTE® Serum Sep Clot Activator tubes (gel tube), and different in the Chromatogram shape, the gel separator may interfere with analysis (Figure 3). Discrepancies in serum samples prepared in tubes with clot activator were detected [12,13].

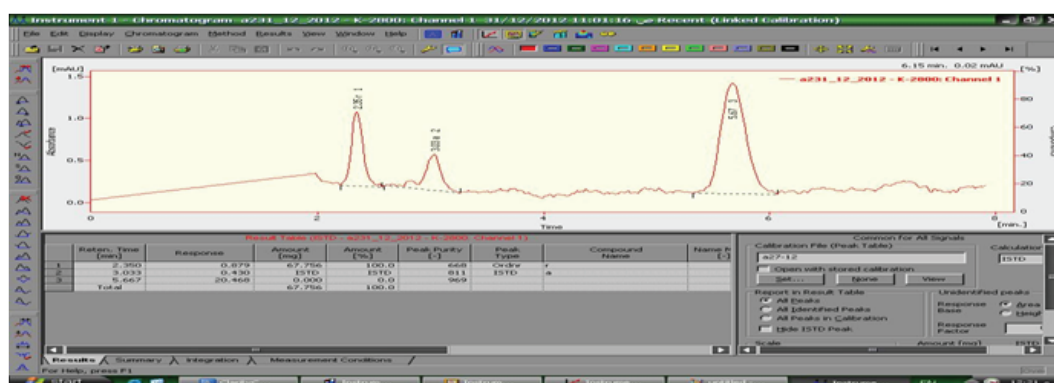


Figure 1: Chromatogram of human serum no additive tube, retinol Concentration 67.7 μ g/dl.

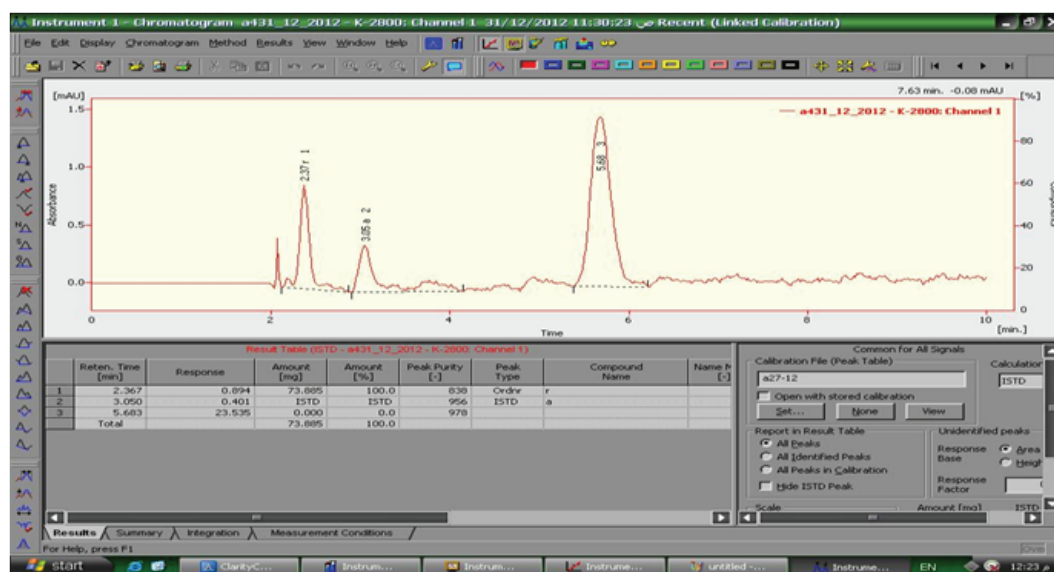


Figure 2: Chromatogram of human plasma, retinol Concentration 73.8 μ g/dl.

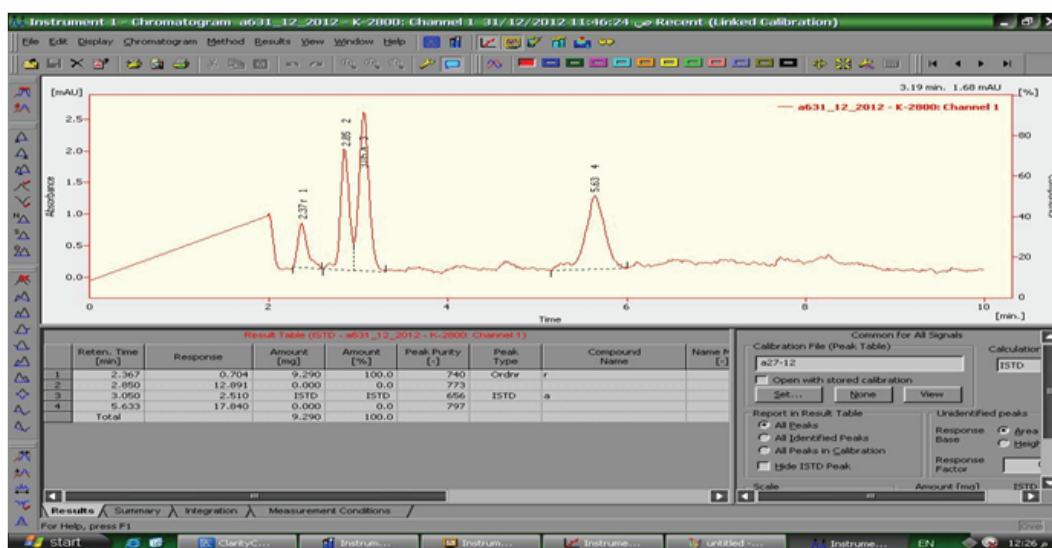


Figure 3: Chromatogram of human serum gel tube, retinol Concentration 9.2 ug/dl.

Discussion

Laboratory test results are dependent on the quality of the specimen submitted. If there is any doubt or question regarding the type of specimen that should be collected, it is imperative that the laboratory is called to clarify the order and specimen requirements.

The pre-analytical steps are needed for blood sampling by using serum or plasma samples and the processing influence vitamin A determination by HPLC required.

The inner walls of the vacutte serum tubes have a special coating of microscopic silica particles to activate the coagulation process. Serum gel tubes contain an inert barrier gel that is present in the bottom of the tube. During centrifugation the barrier gel moves upward to the serum-clot interface, where it forms a stable barrier separating the serum from fibrin and cells.

Limitation

1. Refer to the instrument assay's instructions for use for information on the correct sample material, correct storage and stability.
2. Verify the compatibility prior to use. If there is no assay compatibility, it could lead to false or invalid analysis results.
3. Vitamin D3 determination by HPLC cannot be carried out with all gel tubes without some restrictions (13,14,15).

Conclusion

Tubes are drawn in a specific order to avoid the possibility of erroneous test results caused by carryover of an additive. If Serum tube-with or without clot activator or gel. This tube is either a red top tube or a gold top tube depending on manufacturer and tube additive; it should be drawn as the first tube. We recommended that only serum and EDTA -plasma samples be used for Vitamin A determination by HPLC and cannot be carried out with all gel tubes.

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