Gel Blood Collection Tube Affecting Test Results

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Citation

Abstract
Accurate laboratory testing requires an understanding of the interactions between collection tubes and blood specimens which can adversely affect the accuracy of laboratory test results. The blood collection tubes like plain tube (containing no gel) used for collection of serum for selected chemistry tests, and serum gel tube contains a clot activator and gel separator used for various laboratory tests. Gel blood collection tube is influenced by a number of variables, some of which are controlled by the tube manufacturer, tube material, centrifugation speed and temperature; separator gel may release materials that interfere with analytical assays. This study is an investigation of the effect of gel blood collection tubes on vitamin A retinol, 25-OH vitamin D assays by HPLC method.

1. Introduction

Fig. 1. Gel tube & plain tube (no gel).
“Serum-separator tubes, also known as SST, gel tubes, are widely used in the clinical laboratory for routine collection blood, it contain silica particles as a clot activator and a special gel (the gel is composed of inert components, which are part of a polyester-based proprietary formulation) [1]. The gel forms a physical barrier between serum and blood cells during centrifugation, supernatant serum aspirated directly from the collection tube.

“Gel blood collection tube is influenced by a number of variables, some of which are controlled by the tube manufacturer, tube material, centrifugation speed and temperature; separator gel may release materials that interfere with analytical assays [2].

“Several previous studies from USA, Japan, China, and Iraq have shown the effects of interfering of gel on clinical assay such as: gel may interfere with assays and affecting analyte concentrations stability of blood analytes after storage in BD SST tubes for 12 mo, Clinically significant changes occurred only in 1,25-dihydroxyvitamin D and retinol-binding protein [3].

“Separator gel may add materials, adsorb blood components, or interact with protein and cellular components [4]. “Separator gels lead to a decrease in serum drug concentrations [5]. “Separator gels may release materials (e.g., gel pieces and silicone oil) into the specimens and spuriously interfere with assays [6].

“The separator gel components (SST) were the source of interference in the quantitation of serum testosterone levels [7]. “Blood for 25-OH D measurement is probably best collected into plain tubes without anticoagulants or gels [8].

“Gels have adverse effects on some steroid assays, including HPLC methods for 25-OH D [9]. “Unusual chromatography – especially for 25OHD2 – was noted for some patient samples. An investigation prompted the hypothesis that gel separator in the blood collection tubes may cause interference [10].

“It was noteworthy that some unexpected peaks appeared in blood specimens collected in the particular blood collection tube [11]. “Serum 25OHD in VACUETTE tubes with gel and clot activator, as measured by the Siemens system, produced significantly higher values than did samples collected in VACUETTE tubes with no additives [12].

“Laboratories and tube manufacturers should be aware of the limitation of using any tubes containing gel-separator [13]. “Vitamin D3 determination by HPLC cannot be carried out with all gel tubes [14].

“Further studies have showed that an anomalous result from gel tubes for vitamin D [15]. “A recent blood collection problem in our setting Troponin, Vitamin A, and E [16].

“Blood collection tubes that contain separator gel interfered with the quantification of steroid molecules 17-hydroxyprogesterone and aldosterone by introducing extraneous molecules that interfered with LC-MS/MS analysis [17]. “The serum TT3 concentrations obtained with the SST tubes showed a significant positive proportional difference compared with glass and Vacuette tubes [18].

“Do not use serum separator tubes for therapeutic drug monitoring or toxicological analysis. The plastic serum separator material extracts lipophilic substances (most drugs), resulting in a falsely low drug concentration result. Instead, collect the specimen in a plain red-top tube containing no anticoagulants or preservatives [19].

The aim of the present study is to investigate if gel tubes cause chromatographic interference with vitamin A (retinol) and vitamin D (25OHD3) analysis by HPLC method.

2. Methods

Blood samples were collected from three healthy volunteers into two tube types, gel separator serum tubes, and plain tubes (tubes with no gel).

Blood collected in a plain tube (no gel), allow blood to clot at room temperature for 30 minutes then centrifuge for 10 minutes to separate serum from clot.

Gel separator tube: gently invert the tube several times (eight times). Allow blood to clot at ambient temperature for 20-30 minutes. Centrifuge for 10 minutes to separate serum from clot, If frozen serum is required, pour off serum into plastic screw-cap vial and freeze [20].

Vitamin A assay by HPLC

Assay the concentration of vitamin A, by using HPLC system knauer with a smart line UV detector 2500, pump 1000, and manager 5000. C18 column [250×4.6 mm (I.d.); 5 μm 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. The quantitative results were expressed as ug/dl vs. control and calibrator.

Vitamin D assay by HPLC

Assay the concentration of vitamin D, RP- C18 column (100 x 4.6 mm I.D.; particle size, 5 micron) at a flow-rate of 1 ml/min, the mobile phase was methanol. The eluate was monitored with photodiode-array detector with wavelengths 265 nm. The quantitative results were expressed as ng/dl vs. control and calibrator.

3. Results

Patient samples collected in gel separator tubes showed that;

The chromatogram of retinol and 25OHD were Unusual, they were not similar in shape, not identical the peaks differ in height and there was a new peak present in gel tube sample than the same patient samples collected in plain tubes (no gel) (Fig. 2 and Fig. 3) and (Fig. 4 and Fig. 5)

The chromatography from the gel separator tubes may affect the quantitation of both retinol and 25OHD.
Fig. 2. Chromatogram of patient, serum collected into plain tube, vitamin A had a retention time of 2.3 minutes and 3.0 minutes for the internal standard, retinol concentration ug/dl.

Fig. 3. Chromatogram of the same patient, serum collected into (gel tube), retinol Concentration ug/dl.
A new and unusual peak present between retinol and internal standard and the peaks show differ in height which effect the concentration and vitamin result.

Comparing the chromatogram of vitamin D in serum collected in plain tubes and those collected in gel tubes, the results were obtained as in (Fig3 and Fig4).

**Fig. 4.** Chromatogram of patient, serum collected into plain tube, vitamin D had a retention time of 3.7 minutes and 6.3 minutes for the internal standard, vitamin D concentration ng/ml.

**Fig. 5.** Chromatogram of the same patient, serum collected into (gel tube), vitamin D3 Concentration ng/ml.
A new and unusual peak present and the peaks show differ in heights which effect the concentration and vitamin result.

Tube comparison study for patient serum collected into plain tube (no gel) and gel blood collection tubes show the effects of gel which caused chromatographic interference with retinol and 25OHD and detect the errors which affect the ability of clinical laboratories to produce accurate results.

4. Discussion

Ideally, the gel in blood collection tube should be inert to the specimens collected in.

The gel components in blood collection tube may release materials (gel pieces and silicone oil) into the specimens and cause chromatographic interference with vitamin A (retinol) and vitamin D (25OHD) by the HPLC method compared to plain blood collection tube (no gel).

“Using gel vials for blood collection might caused disturbed chromatograms caused by ingredients of the gel. We recommend using EDTA plasma or serum vials without gel [21].”

Finally, evaluation of blood collection tubes by reference clinical laboratories should be done to help in detecting tube-related errors and interferences in test results which can adversely influence patient outcomes and decrease laboratory efficiency.

Table 1. shows blood collection tubes-majority of laboratories collect blood either in plain tubes or gel tubes. For 25-OH vitamin D analysis;

<table>
<thead>
<tr>
<th>Medical laboratories</th>
<th>Collection Container/Tube Vitamin A (Retinol)</th>
<th>Collection container/Tube 25-OH vitamin D</th>
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<td>Preferred; Plain tube</td>
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Specimen Container;
Plain tube, red top tube, (No-Gel)
Gel blood collection tube, gel separation tube, serum gel, SST
5. Conclusions

Gel collection tubes caused chromatographic interference with vitamin A (Retinol) and vitamin D (25OHD) quantitation by the HPLC method compared to serum collection tubes by plain tube (containing no gel).

Gel blood collection tubes may adversely influence patient outcomes, patient treatment, patient monitoring, patient diagnosis and decrease laboratory efficiency, and affect the clinical decision.

Standardization of collection tube procedure will be an important element in accurate analysis.

References


[19] Quest Diagnostics; Specimen Collection Tubes www.questdiagnostics.com/.../specimen_collection_tub"

[20] UC Irvine Medical Center, Department of Pathology, 101 The City Drive, Orange, CA 92868. http://www.pathology.uci.edu/services/specimen-containers.asp