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Effect of Blood Collection Tubes on Vitamin D Immunoassay Results

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Dear Editor,

Blood collection tubes are generally considered inert specimen carriers but can potentially induce exogenous interference. Blood collection tubes consist of rubber stoppers, tube-wall materials, separator gels, clot activators or anticoagulants, and surfactants, all of which can interfere with analytical assays [1]. We observed apparent inconsistency in Atellica IM Vitamin D Total assay results (Siemens Healthineers, Forchheim, Germany) across different tubes; therefore, we examined the effect of blood collection tubes on 25-hydroxyvitamin D (25(OH)D) as part of an end-user verification, with approval from the institutional review board (IRB) of Seoul St. Mary's Hospital (IRB No. KC23DSSF0058).

The following four tubes were examined for potential assay interference: Greiner Bio-One Vacuette 8-mL serum-separator tube (SST) (Greiner Vacuette; Cat. #455071KR, lot #A2309376; Greiner Bio-One, Kremsmünster, Austria), Becton Dickinson (BD) Vacutainer 8.5-mL SST (BD Vacutainer; Cat. #367528, lot #2237485; BD, Franklin Lakes, NJ, USA), AB Medical V-Tube 8-mL tube with clot activator and gel (V-tube; Cat. #301802, lot #8337002; AB Medical, Seoul, Korea), and AB Medical VQ-Tube 8-mL with clot activator with thrombin and gel (VQ-tube; Cat. #B0180B, lot #8B41002; AB Medical). Serum samples from four apparently healthy volunteers (two men and two women; age range, 26–49 yrs) were collected in each of the abovementioned tubes and analyzed for 25(OH)D using the Atellica IM Vitamin D Total assay. Samples collected in Greiner Vacuette tubes exhibited higher values (mean bias, 9.95 ng/mL; range, 4.56–12.34 ng/mL) than those collected in other tubes (Table 1, Fig. 1). This increase would lead to incorrect classification of all four individuals as not deficient in vitamin D, despite having a 25(OH)D level <20 ng/mL (the recommended cutoff value for vitamin D deficiency) [2]. When the samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with PerkinElmer (Wallac Oy, Turku, Finland) kits, no tube-induced bias (<5%) was noted. This LC-MS/MS method was certified by the Centers for Disease Control and Prevention Vitamin D Standardization and Certification Program, as previously documented [3].

The tube-induced bias was further examined using the Roche Elecsys Vitamin D Total III (Roche Diagnostics International Ltd,. Rotkreuz, Switzerland) and Atellica IM Vitamin D Total assays. Additionally, serum samples of three other apparently healthy individuals were collected in both Greiner Vacuette and VQ tubes for analysis. Samples collected in Greiner Vacuette tubes exhibited higher 25(OH)D values (mean bias, 6.22 ng/mL) than those

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Table 1. Comparison of 25-hydroxyvitamin D in samples from healthy volunteers collected in different tubes and analyzed using different assays

No. sample	25-hydroxyvitamin D, ng/mL									
	Siemens Atellica IM Vitamin D Total assay				Roche Elecsys Vitamin D Total III		LC-MS/MS			
	Greiner Vacuette	BD Vacutainer	V-tube	VQ-tube	Greiner Vacuette	VQ-tube	Greiner Vacuette	BD Vacutainer	V-tube	VQ-tube
1	20.8	10.8	9.1	7.8	ND	ND	6.9	7.0	6.9	7.0
2	26.4	16.1	14.5	14.5	ND	ND	16.1	16.4	16.7	16.8
3	29.2	15.2	18.5	16.9	ND	ND	18.0	18.1	18.3	17.8
4	40.3	38.6	35.8	32.8	ND	ND	32.5	32.7	33.1	33.4
5	28.4	ND	ND	20.9	22.0	22.9	ND	ND	ND	ND
6	10.4	ND	ND	5.4	7.6	8.8	ND	ND	ND	ND
7	18.0	ND	ND	11.9	15.8	16.4	ND	ND	ND	ND

Abbreviations: LC-MS/MS, liquid chromatography-tandem mass spectrometry; Greiner Vacuette, Greiner Bio-One Vacuette 8-mL serum-separating tube; BD Vacutainer, Becton Dickinson Vacutainer 8.5-mL serum-separating tube; V-tube, AB Medical V-Tube 8-mL tube with clot activator and gel; VQ-tube, AB Medical VQ-Tube 8-mL with clot activator with thrombin and gel; ND, not determined.

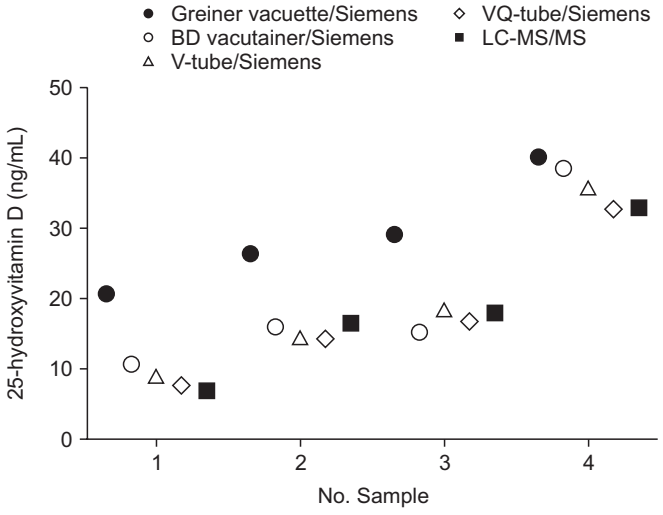

Fig. 1. Positive interference for 25-hydroxyvitamin D observed with serum collected in Greiner Vacuette serum-separator tubes and measured using the Siemens Atellica IM assay. The positive interference is more significant for lower 25-hydroxyvitamin D levels than for higher levels.

Abbreviations: BD, Becton Dickinson; Greiner Vacuette, Greiner Bio-One Vacuette 8-mL serum-separating tube; BD Vacutainer, Becton Dickinson Vacutainer 8.5-mL serum-separating tube; V-tube, AB Medical V-Tube 8-mL tube with clot activator and gel; VQ-tube, AB Medical VQ-Tube 8-mL with clot activator with thrombin and gel; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

collected in VQ tubes when analyzed with the Siemens Atellica IM system. Conversely, no significant bias was observed between the two tubes with the Roche Elecsys Vitamin D Total III assay.

To our knowledge, this is the first report of increased 25(OH)D levels resulting from blood collection in Greiner Vacuette tubes in conjunction with the Atellica IM Vitamin D Total assay. Although the reasons for this observation are not fully understood, tube surfactants, which tend to vary among manufacturers, may affect the vitamin D assay. Notably, tube surfactants are employed to minimize adsorption to the tube wall; however, at sufficiently high concentrations, they may lead to the desorption of antibodies from the solid phase, among other nonspecific effects [4]. The Atellica IM Vitamin D Total assay is a competitive immunoassay that uses anti-fluorescein-labeled mouse monoclonal antibodies covalently bound to paramagnetic particles, a fluorescein-labeled vitamin D analog, and an acridinium ester-labeled anti-25(OH)D mouse monoclonal antibody. Hence, the potential of the surfactant to induce the desorption of antibodies from the solid phase may have lowered the chemiluminescent signal and falsely increased 25(OH)D levels. Notably, a similar bias has been reported for Siemens ADVIA Centaur XP for 25(OH)D when using Vacuette tubes containing a clot activator with or without gel compared to Vacuette tubes with no additives [5, 6]. Because the reagent formulations used in the Atellica IM Analyzer and the ADVIA Centaur system are similar, their interference mechanism may be the same.

Our study is limited by its small sample size. Nevertheless, examining various tubes with specimens obtained from healthy individuals is not readily accessible in routine practice, and our findings clearly show the impact of additives in blood collection tubes on vitamin D immunoassay results. Detecting and preventing tube additive-induced interference poses a unique challenge for clinical laboratories. Timely recognition is difficult be-

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cause routine QC typically does not include the preanalytical phase, including sample collection and processing in blood collection tubes, in which contact with the tube additives occurs [7]. Therefore, in addition to performing a thorough comparative study, clinical laboratories should be vigilant of any possible changes in assay performance when using new lots and/or different tubes. Furthermore, manufacturers must validate the safety and efficacy of their blood collection tubes through analytical and clinical evaluations, preferably under conditions of maximal interference across all analytical platforms/assays.

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AUTHOR CONTRIBUTIONS

Chae H and Oh E-J designed the study. Lee S and Choi A-R performed the analysis and collected the data. Cho S-E provided the LC-MS/MS results and interpretation. Chae H wrote the initial draft of the manuscript.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. Bowen RAR, Hortin GL, Csako G, Otaez OH, Remaley AT. Impact of blood collection devices on clinical chemistry assays. Clin Biochem 2010;43:4-25.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30.
3. Seo JD, Lee JH, Cho SE, Song J, Lee YW, Yun YM. Evaluation of test standardization of 25-OH vitamin D immunoassays. Lab Med Online 2024;14:8-16.
4. Bowen RAR, Chan Y, Ruddel ME, Hortin GL, Csako G, Demosky SJ Jr., et al. Immunoassay interference by a commonly used blood collection tube additive, the organosilicone surfactant silwet L-720. Clin Chem 2005;51:1874-82.
5. Yu S, Cheng X, Fang H, Zhang R, Han J, Qin X, et al. 25OHD analogues and vacuum blood collection tubes dramatically affect the accuracy of automated immunoassays. Sci Rep 2015;5:14636.
6. Yu S, Zhou W, Cheng X, Fang H, Zhang R, Cheng Q, et al. Blood collection tubes and storage temperature should be evaluated when using the Siemens ADVIA Centaur XP for measuring 25-hydroxyvitamin D. PLoS One 2016;11:e0166327.
7. Kricka LJ, Park JY, Senior MB, Fontanilla R. Processing controls in blood collection tubes reveals interference. Clin Chem 2005;51:2422-3.