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Therapeutic concentrations of hydroxocobalamin interferes with several spectrophotometric assays on the Beckman Coulter DxC and AU680 chemistry analyzers

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ABSTRACT

Background: High doses of hydroxocobalamin (OHCob) are used to treat cyanide poisoning and cardiac complications. Since OHCob absorbs at multiple wavelengths often used in colorimetric assays, spurious laboratory results are likely to occur. The objective of this study was to examine interference caused by OHCob in colorimetric assays measured using the Beckman Coulter DxC and AU680.

Methods: OHCob was dissolved in water and spiked into pooled "healthy" and "unhealthy" patient samples at two different concentrations (0.15 and 1.5 mg/mL). Spiked and unspiked samples were analyzed on both instruments and bias was calculated. A total of 23 analytes were tested on the DxC and 27 analytes were tested on the AU680. For analytes showing a bias ≥10%, OHCob was titrated from 0.2–1.5 mg/mL.

Results: The following analytes were affected on the DxC and AU680: alanine aminotransferase, amylase, total bilirubin, cholesterol, creatine kinase, creatinine, magnesium, uric acid. Direct bilirubin, iron, phosphate, total protein and triglycerides were only affected on the DxC. Biases observed were positive or negative and fixed or proportional.

Conclusions: Between the DxC and AU680, several analytes were affected at therapeutic OHCob concentrations. Hence, it is important for laboratories to know how their instruments are affected, and for clinicians to alert the lab when these samples are expected.

1. Introduction

Hydroxocobalmin (OHCob), a precursor of cyanocobalamin (vitamin B12) is an FDA approved antidote (Cyanokit®) used to treat cyanide poisoning. It detoxifies by binding cyanide with a high affinity and forming the non-toxic compound, cyanocobalamin, which is renally cleared [1]. More recently, OHCob has been used therapeutically to treat vasoplegic syndrome (VS), a common complication of cardiopulmonary bypass (CPB). VS is characterized by significant hypotension, high to normal cardiac output and low systemic vascular resistance [2]. While the exact mechanism of how this occurs is not known, it is hypothesized to be a combination of vasodilator activation and vasopressor resistance [2]. One of the dysregulated pathways leading to VS is nitric oxide (NO) homeostasis, where NO acts as a powerful vasodilator. OHCob is thought to bind NO and inhibit nitric oxide synthase and guanylate cyclase, resulting in a significant rise in blood pressure [3,4].

Typically, a 5 g dose is administered in either cyanide poisoning or VS. This dose is sufficient to cause a pink discoloration of mucous membranes, skin, serum and urine [5–7]. Our institution recently encountered a significant rise in the use of hydroxocobalamin to treat VS after a CPB. Both serum and urine samples collected from these patients have a distinct red discoloration (Fig. 1), but are not flagged by automated analyzers because of differences in the absorbance spectrums of hemoglobin and OHCob [8,9].

OHCob is a colored compound that absorbs at wavelengths (274, 351, 500 and 526 nm) often used in colorimetric assays [6]. As such, spectral interferences may produce spurious laboratory values when patients are treated with high doses of OHCob, which is supported by several reports [6,7,10]. However, the mechanism of interference extends beyond just a spectral effect as hydroxocobalamin is thought to interfere with chemical reactions within the tests [10]. As a result, it is difficult to predict which tests are affected and to what extent. The specific instrumentation/assays used in our laboratory had not been assessed for OHCob interference and we therefore aimed to examine OHCob interference in colorimetric assays measured on the Beckman Coulter DxC and AU680.

2. Materials and methods

2.1. Study approval

This study was performed as part of ongoing quality assurance/quality improvement studies at the University of Washington, Department

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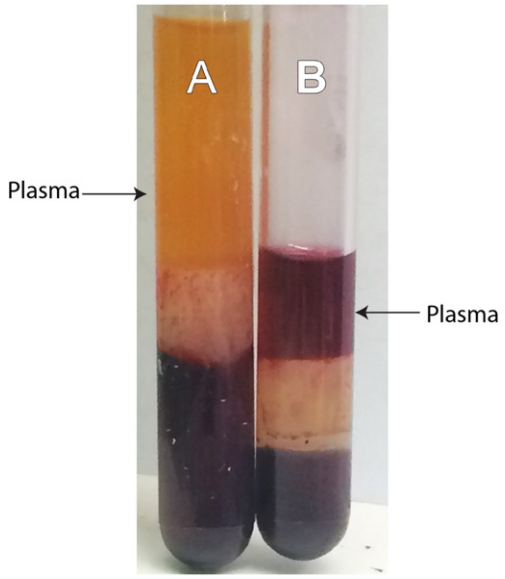

Fig. 1. Plasma appearance before (A) and 6 h after (B) administration of 5 g of OHCOb. The plasma portion of the sample is labeled. Notice the distinct red discoloration in sample B.

of Laboratory Medicine and as such, was granted an exemption from the UW Institutional Review Board.

2.2. Reagents and instrumentations

Hydroxocobalamin (OHCob) was purchased from Sigma Aldrich, USA (cat# H1428000) and used to prepare a 10 mg/ml aqueous stock solution. All chemistry quantifications were performed on the Beckman Coulter DxC or AU680 instruments at the University of Washington Medical Center. The methods and the measurement wavelengths for each analyte on the 2 instruments are listed in Supplemental Table 1.

2.3. OHCob interference

"Healthy" and "unhealthy" plasma pools (n = 1–2) were prepared using residual, de-identified specimens and frozen until further use. "Healthy" was defined as sample pools with analyte concentrations that were within the reference interval. "Unhealthy" was defined as sample pools with some or all of the following analytes elevated with values ≥1.5× the upper end of the reference range: aspartate aminotransferase (AST), amylase, alanine aminotransferase (ALT), direct bilirubin, total bilirubin, creatine kinase, and creatinine. Triglycerides were defined as "unhealthy" if the concentration was >150 mg/dL. Table 1 lists the pool concentrations of the analytes investigated. Sample pools were thawed and spiked with 0.15 mg/mL OHCob, 1.5 mg/mL OHCob or an equivalent volume of water. The low (0.15 mg/ml) and the high (1.5 mg/mL) concentrations of OHCob were chosen based on expected therapeutic levels (0.4–1.3 mg/mL) after a 5 g dose given intravenously in the treatment of cyanide poisoning [11,12]. The following routine chemistry analytes were measured in spiked and unspiked pools on both platforms: albumin, alkaline phosphatase, alanine aminotransferase (ALT), amylase, direct bilirubin (Dbil), total bilirubin (Tbil), cholesterol, creatine kinase (CK), creatinine, direct low density lipoprotein (D-LDL), gamma-glutamyl transferase (GGT), high density lipoprotein (HDL), iron, lactate dehydrogenase (LD), lipase, magnesium (Mg), phosphate, total protein, transferrin, transthyretin, triglycerides and uric acid. Aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca) and glucose were measured only on the AU680.

Table 1
Concentration of analytes in unspiked "healthy" and "unhealthy" pools.

	"Healthy"				"Unhealthy"			
	Pool 1		Pool 2		Pool 1		Pool 2	
Analyte	DxC	AU680	DxC	AU680	DxC	AU680	DxC	AU680
ALT	30	23	--	15	--	--	--	--
ALT	18	23	--	22	199	199	--	252
Amylase	95	31	68	65	--	--	--	--
Amylase	63	29	--	22	177	111	--	--
AST	--	52	--	19	--	--	--	--
AST	--	50	--	52	--	603	--	315
Bilirubin (Direct)	0.1	0.16	--	--	--	--	--	--
Bilirubin (Direct)	0.1	0.17	--	0.15	4.7	4.34	--	5.76
Bilirubin (Total)	0.6	0.6	--	0.4	--	--	--	--
Bilirubin (Total)	0.5	0.6	--	0.5	7	7.5	--	10.3
Cholesterol	127	153	--	120	--	--	--	--
Cholesterol	127	146	106	90	--	--	--	--
Creatine Kinase	54	42	54	129	--	--	--	--
Creatine Kinase	52	40	--	--	620	508	--	430
Creatinine	1.03	0.73	--	0.96	--	--	--	--
Creatinine	0.87	0.6	--	0.7	2.72	1.3	--	5.4
Iron	51	55	93	--	--	--	--	--
Iron	84	50	--	85	--	--	--	--
Magnesium	1.6	1.6	--	1.7	--	--	--	--
Magnesium	1.8	1.5	--	1.6	--	--	--	--
Phosphate	3.6	3.1	--	3.4	--	--	--	--
Phosphate	3.0	2.7	--	2.9	4.7	--	--	--
Total Protein	6.1	6.2	5.8	--	--	--	--	--
Total Protein	5.5	5.8	--	--	--	--	--	--
Triglycerides	77	96	--	73	--	--	--	--
Triglycerides	55	94	--	--	157	153	--	--
Uric Acid	5.3	5.2	3.9	5.4	--	--	--	--
Uric Acid	3.7	4.7	5.7	3.9	--	--	--	--

Analytes shaded in gray were the pools used to spike in 0.15 mg/mL OHCob. All others were pools used to spike in 1.5 mg/mL OHCob.
–; not quantified.