Effects of Delayed Specimen Processing and Freezing on Serum Concentrations of Selected Nutritional Indicators

Bakary Drammeh, Phuong Hong Nguyen, Rosemary L Schleicher, Christine M Pfeiffer, Ram Jain, Mindy Zhang
Centers for Disease Control and Prevention, Atlanta, GA

Introduction

Micronutrient deficiencies are an important public health problem in developing countries. To assess their prevalence, blood is typically collected during national micronutrient surveys in a rural setting. It is almost impossible to immediately process and transport blood samples each day to a central lab. Furthermore, deep freezers and dry ice for transportation of frozen specimens are often unavailable. As a consequence, there are delays in specimen processing and freezing.

The CDC Nutritional Biomarkers Laboratory is often asked whether a delay in blood processing and freezing of serum affects certain lab tests. Previous reports on stability of nutritional indicators (1-4) do not mimic practical conditions in developing country settings. We therefore investigated the effects of delayed blood processing and serum freezing on nutritional indicators. To simulate a delay in whole blood processing, we kept whole blood at 32ºC for up to 3 days. To mimic delayed serum shipping, we assumed that serum transportation leads to a breakdown in cold chain and temperature fluctuates around 11ºC for up to 14 days.

Objective

The objective of this research project was to determine if different pre-analytical factors mimicking delayed specimen processing and freezing affect the concentrations of iron indicators and selected vitamins.

Method

Venous blood was collected from each of 35 volunteers into 11 red-top vacutainer tubes half of which were immediately stored as whole blood at 32ºC for 1, 2 and 3 days after which time the sera separated. The other red-top vacutainer tubes were centrifuged 30-90 minutes after collection. One serum aliquot was immediately frozen at -70ºC (baseline); the other aliquots were stored at 11ºC for 2, 7, 10 and 14 days. All serum samples were stored after their particular treatment at -70ºC until analyzed.

Biomarkers were assayed using different methods: Ferritin and transferrin receptor using immuno-turbidimetry (IT) and enzyme-linked immunoassay (ELISA); vitamin D using radioimmuno-assay (RIA); and folate and B12 using radioprotein-binding assay (RPBA). All treatment conditions from one subject were analyzed in one assay to minimize run-to-run analytical imprecision. We calculated means and standard errors (SE) for each biomarker and each treatment condition. We also calculated mean changes (absolute and relative) for each treatment condition against baseline and performed two tailed paired t-tests using SAS software to determine if differences were significant ($p < 0.05$).

Results

Table 1. Mean concentrations of nutritional indicators in stored serum or serum prepared from stored whole blood

Analyte (units)	Method	Cut-off value	Baseline mean Concentration (SE)	Mean concentration in serum stored at 11ºC (SE)				Mean concentration in whole blood stored at 32ºC (SE)		
				2 d	7 d	10 d	14 d	1 d	2 d	3 d
Ferritin (ng/mL)	Roche (IT)	15	92.3 (12)	92.9 (12)	91.8 (12)	92.4 (12)	93.5 (13)	97.8 (12)	97.8 (12)	96.1 (11)
TFR (mg/L)	Roche (IT)	4	3.3 (0.2)	3.3 (0.2)	3.3 (0.2)	3.3 (0.2)	3.3 (0.2)	3.5 (0.2)	3.4 (0.2)	3.2 (0.2)
TFR (mg/L)	Ramco (ELISA)	8	5.3 (0.3)	5.2 (0.3)	5.4 (0.3)	5.6 (0.3)	5.7 (0.3)	5.6 (0.2)	5.5 (0.3)	5.1 (0.3)
Vitamin A (µg/dL)	HPLC/UV	20	61.4 (2.2)	61.4 (2.1)	62.2 (2)	61.9 (2)	63.1 (2)	61.2 (2)	59.9 (2)	55.3 (2)
Vitamin E (µg/dL)	HPLC/UV	500	1125 (47)	1132 (50)	1139 (49)	1138 (49)	1161 (51)	1158 (50)	1156 (49)	1113 (48)
Vitamin D, 25-hydroxy (ng/mL)	Diasorin (RIA)	15	22.7 (1.2)	22.5 (1.1)	24.2 (1.5)	23.3 (1.2)	23.4 (1.4)	22.1 (1.1)	22.5 (1.3)	22.2 (1.2)
Folate (ng/mL)	Biorad (RPBA)	3	16.1 (2)	15.5 (2)	14.3 (2)	14.0 (2)	11.7 (1.4)	13.6 (2)	11.7 (2)	11.8 (2)
Vitamin B12 (pg/mL)	Biorad (RPBA)	200	525 (67.4)	517 (65)	511 (65)	518 (69)	509 (66)	659 (64)	625 (75)	537 (63)
Pyridoxal-5'-phosphate (nmol/L)	HPLC/FD	30	85.7 (13)	81.0 (12)	79.5 (12)	79.3 (12)	77.1 (12)	82.7 (12)	83.5 (12)	91.4 (14)
4-Pyridoxic acid (nmol/L)	HPLC/FD	none	111 (29)	112 (29)	112 (29)	115 (30)	121 (32)	114 (29)	114 (28)	111 (27)

Table 2. Mean changes in concentrations of nutritional indicators in stored serum or serum prepared from stored whole blood[1]

Analyte (units)	Serum stored at 11ºC				Whole blood stored at 32ºC		
	2 d	7 d	10 d	14 d	1 d	2 d	3 d
Ferritin (ng/mL)	0.6 (0.8) 2% (-0.6, 4.7)	1 (0.8) 0.9% (-1.3, 3)	0.1 (1) 2.4% (-1.2, 6)	1.4 (1.1) 1.7% (-2.2, 5.6)	5.5 (0.9) * 9.4% (6, 13)	5.5 (1) * 8.4% (4.2, 13)	5.2 (1.4) * 13.2% (6.3, 20)
TFR (mg/L) Roche	0 (0) 0.5% (-0.6, 1.5)	0 (0) 0.6% (-0.5, 1.6)	0 (0) 0.7% (-0.2, 1.5)	0 (0) 0.7% (-0.4, 1.7)	0.2 (0) * 5.3% (2.8, 7.8)	0.1 (0) * 4.3% (2.1, 6.6)	-0.1 (0) -1.5% (-3.8, 0.8)
TFR (mg/L) Ramco	-0.1 (0.1) -3.1% (-7.8, 1.5)	0.1 (0.1) 2% (-2.7, 6.7)	0.3 (0.1) * 6.5% (2.1, 11)	0.4 (0.1) * 7.8% (4,12)	0.3 (0.1) * 6.6% (2.4, 11)	0.1 (0.1) 3.5% (-1.1, 8.1)	-0.3 (0.1) * -5.5% (-9.7, -1.4)
Vitamin A (µg/dL)	0.1 (0.5) 0.3% (-1, 1.6)	0.8 (0.6) 1.5% (0, 3)	0.5 (0.4) 0.9% (-0.2,2)	1.7 (0.5) * 3% (1.6, 4.4)	-0.2 (0.5) -0.3% (-1.5, 0.9)	-1.5 (0.5) * -2.4% (-3.7, -1.1)	-6.1 (0.7) * -9.9% (-12, -8)
Vitamin E (µg/dL)	7.1 (4.1) 0.4% (-0.2, 1.1)	15.3 (4.8) * 1.3% (0.5, 2.2)	14 (3.9) * 1.2% (0.5, 1.8)	36.2 (8.3) * 3.1% (1.8, 4.3)	34 (5.7) * 3% (2,3.8)	31.7 (5.7) * 2.8% (2,3.7)	-11.7 (8.9) -1.1% (-2.7, 0.6)
Vitamin D, 25-hydroxy (ng/mL)	-0.2 (0.4) -0.2% (-4, 3.5)	1.5 (0.7) * 6.6% (1.7, 11)	0.4 (0.5) 3.0% (-1.5, 7.5)	0.7 (0.6) 3.1% (-1.3, 7.4)	-0.6 (0.6) -1.3% (-6.1, 3.5)	-0.2 (0.7) 0.5% (-5, 5.9)	-0.5 (0.7) -0.4% (-6.3, 5.4)
Folate (ng/mL)	-0.6 (0.5) -3% (-5.4, -0.3)	-1.8 (0.6) * -10.4% (-13, -7.3)	-2.1 (0.5) * -13.6% (-17, -10)	-4.4 (0.8) * -25.3% (-28, -22)	-2.5 (0.6) * -17.1% (-22, -13)	-4.4 (0.6) * -28.8% (-33, -24)	-4.3 (0.7) * -27.6% (-32, -23)
Vitamin B12 (pg/mL)	-7.5 (5.4) -0.8% (-3, 1.1)	-13.5 (6.3) * -2.2% (-4.3, 0.0)	-7.3 (6.3) -1.4% (-3.6, 0.8)	-15.7 (6.5) * -2.4% (-4.7, -0.1)	134 (15) * 31.4% (26,36)	100 (10.5) * 21.5% (18, 25)	12.1 (10.2) 4.2% (0.9, 7.4)
Pyridoxal-5'-phosphate (nmol/L)	-4.7 (1.4) * -4.3% (-6,-2.7)	-6.2 (2) * -1.5% (-8.1, 5.1)	-8.7 (2.2) * -7.4% (-10, -5)	-11.5 (2.8) * -11.7% (-14, -9)	-3.0 (1.7) -1.9% (-4.5, 0.8)	-2.2 (2.5) 0.5% (-3, 4,1)	5.7 (2.8) * 9% (3.6, 14.5)
4-Pyridoxic acid (nmol/L)	1.1 (0.4) * 1% (0.1, 1.9)	1.6 (0.5) * 3.4% (0.8, 6)	1.6 (0.5) * 2.5% (1.3, 3.8)	4.6 (1.3) * 4.8% (3, 6.5)	3.5 (1) * 10.9% (7.6, 14)	3 (1.6) 13.2% (9.3, 17)	0.4 (2.3) 12.6% (7.8, 18)

[1] Absolute change (SE) and relative change (95% confidence interval) from baseline

* Mean change is significantly different from baseline ($p < 0.05$)

Summary

Ferritin
- Serum stored at 11ºC: No changes up to 14 days.
- Whole blood stored at 32ºC: Increased 9% after 1 day.

Transferrin receptor by Roche
- Serum stored at 11ºC: No changes up to 14 days.
- Whole blood stored at 32ºC: Increased 5% after 1 day.

Transferrin receptor by Ramco
- Serum stored at 11ºC: No changes up to 7 days.
- Whole blood stored at 32ºC: Increased 7% after 1 day.

Vitamin A
- Serum stored at 11ºC: No changes up to 10 days.
- Whole blood stored at 32ºC: No changes up to 1 day, but decreased thereafter.

Vitamin E
- Serum stored at 11ºC: No changes up to 2 days.
- Whole blood stored at 32ºC: Increased 3% after 1 day.

Vitamin D
- Serum stored at 11ºC: No changes up to 2 days.
- Whole blood stored at 32ºC: No changes up to 3 days.

Folate
- Serum stored at 11ºC: No changes up to 2 days.
- Whole blood stored at 32ºC: Large decreases of ~20% after 1 day.

Vitamin B12
- Serum stored at 11ºC: No changes up to 2 days.
- Whole blood stored at 32ºC: Large increases of ~30% after 1 day.

Vitamin B6

Pyridoxal-5'-phosphate
- Serum stored at 11ºC: Moderate decreases of 5% after 2 days.
- Whole blood stored at 32ºC: Increased 9% after 3 days.

4-Pyridoxic acid
- Serum stored at 11ºC: Minor increase of 1% after 2 days.
- Whole blood stored at 32ºC: Increased 11% after 1 day.

References

1. Key T, Oakes S, Davey G, Moore J, Edmond LM, McLoone UJ, et al. Stability of vitamins A, C, and E, carotenoids, lipids, and testosterone in whole blood stored at 4 degrees C for 6 and 24 hours before separation of serum and plasma. Cancer Epidemiol Biomarkers Prev 1996: 5: 811-4
2. Hankinson SE, London SJ, Chute CG, Barbieri RL, Jones L, Kaplan LA, et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989;35
3. Giltay EJ, Geleijnse JM, Schouten EG, Katan MB, Kromhout D. High stability of markers of cardiovascular risk in blood samples. Clin Chem 2003;49:652-5.
4. Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem 1998; 44:1325-33.

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