

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

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## 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 32°C 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 11°C 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 32°C 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 -70°C (baseline); the other aliquots were stored at 11°C for 2, 7, 10 and 14 days. All serum samples were stored after their particular treatment at -70°C until analyzed.

Biomarkers were assayed using different methods: Ferritin and transferrin receptor using immuno-turbidimetry (IT) and enzyme-linked immunoassay (ELISA); vitamin D using radioimmunoassay (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 11°C (SE)				Mean concentration in whole blood stored at 32°C (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<sup>1</sup>

Analyte (units)	Serum stored at 11°C				Whole blood stored at 32°C		
	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)	0.3 (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) -3% (-5.4, -0.3)	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% (-5.9, 5.9)	-0.5 (0.7) -0.4% (-6.3, 5.4)
Folate (ng/mL)	-0.6 (0.5) -3% (-5.4, -0.3)	-1.9 (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)	-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, -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, 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)

<sup>1</sup> 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 11°C: No changes up to 14 days.
- Whole blood stored at 32°C: Increased 9% after 1 day.

### Transferrin receptor by Roche

- Serum stored at 11°C: No changes up to 14 days.
- Whole blood stored at 32°C: Increased 5% after 1 day.

### Transferrin receptor by Ramco

- Serum stored at 11°C: No changes up to 7 days.
- Whole blood stored at 32°C: Increased 7% after 1 day.

### Vitamin A

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

### Vitamin E

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

### Vitamin D

- Serum stored at 11°C: No changes up to 2 days.
- Whole blood stored at 32°C: No changes up to 3 days.

### Folate

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

### Vitamin B12

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

### Vitamin B6

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

### 4-Pyridoxic acid

- Serum stored at 11°C: Minor increase of 1% after 2 days.
- Whole blood stored at 32°C: Increased 11% after 1 day.

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## Acknowledgments

CDC Nutritional Biomarkers Laboratory staff are gratefully acknowledged  
Battelle Memorial Institute; Columbus, OH