

SUPPLEMENTAL INFORMATION

Biomonitoring method for the analysis of chromium and cobalt in human whole blood using inductively coupled plasma - kinetic energy discrimination - mass spectrometry (ICP-KED-MS)

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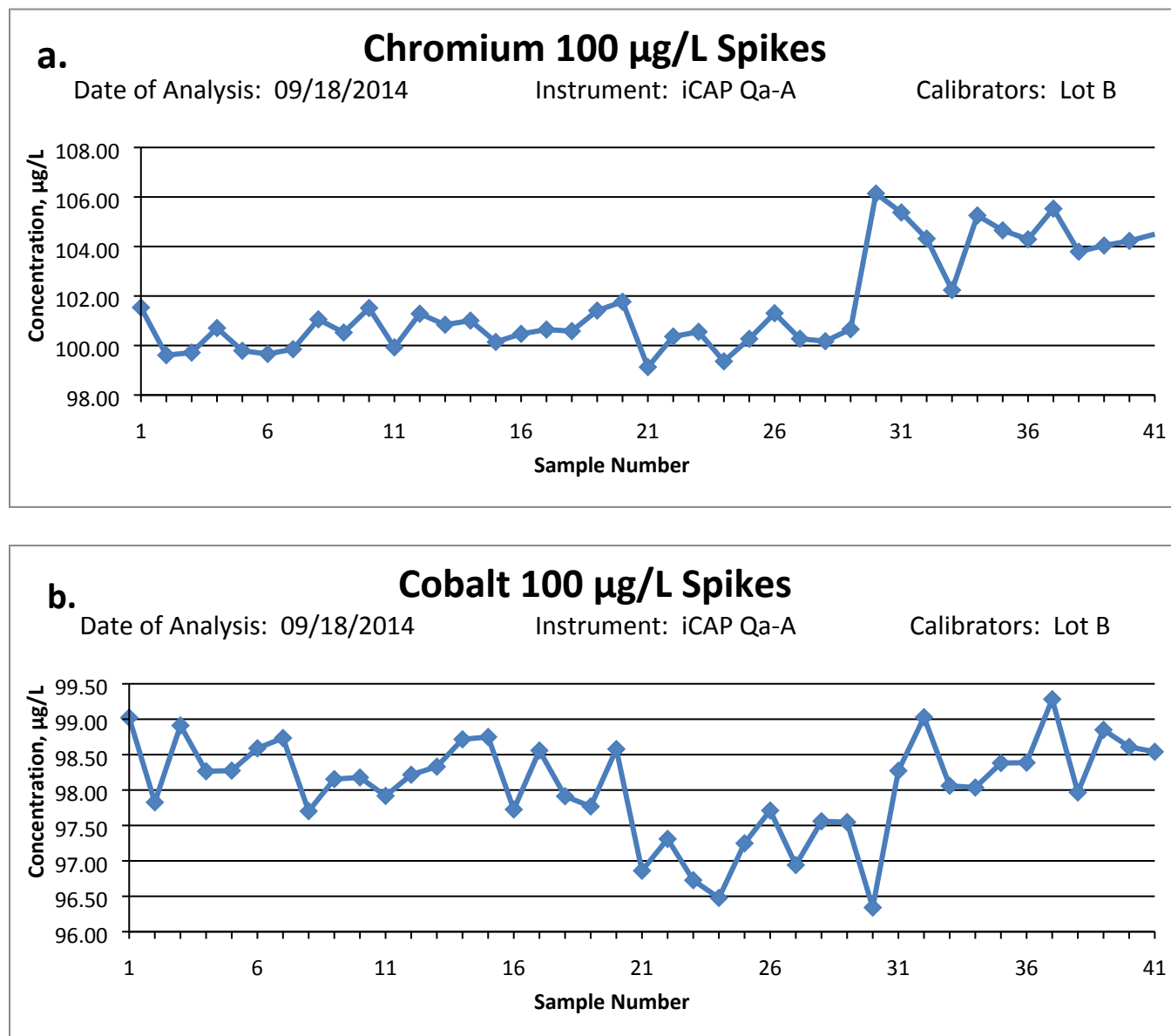
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Center for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, or the U.S. Centers for Disease Control and Prevention

Events	Actions
On Probe Down	FAST Vacuum 1 - On
On Probe Down	FAST Valve 1 Load
Probe in Sample at 4s	FAST Valve 1 Inject
Probe in Sample at 3s	Move Rinse: R1=2s & R2=2s
Rinse Completed	Probe Up
On Rinse	FAST Valve 1 Load
On Rinse at 0.5s	Move Rinse: R1=0s & R2=2s
On Rinse at 12.0s	FAST Valve 1 Inject
On Rinse at 13.0s	FAST Valve 1 Load
Rinse completed	Move To Next

Table S1. ESI SC-4Q FAST Method Parameters

Carryover

Figure S1a-1d. Cr and Co responses (intensities and concentration) for alternating 100 µg/L Cr/Co blood samples and aqueous blanks over time (~3.5hrs.) used to calculate optimum number of samples per run.

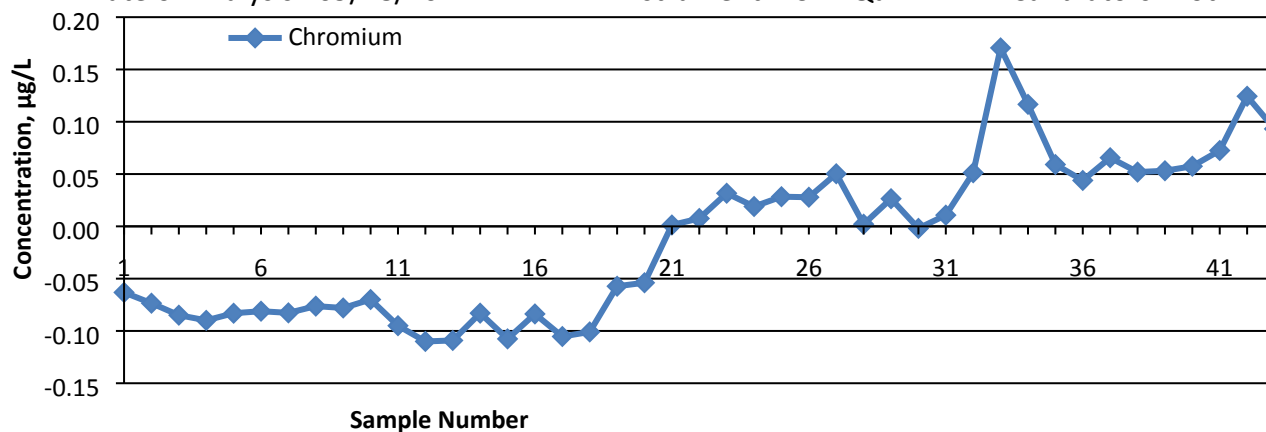


c. Chromium Aqueous Blank Data (Concentrations)

Date of Analysis: 09/18/2014

Instrument: iCAP Qa-A

Calibrators: Lot B

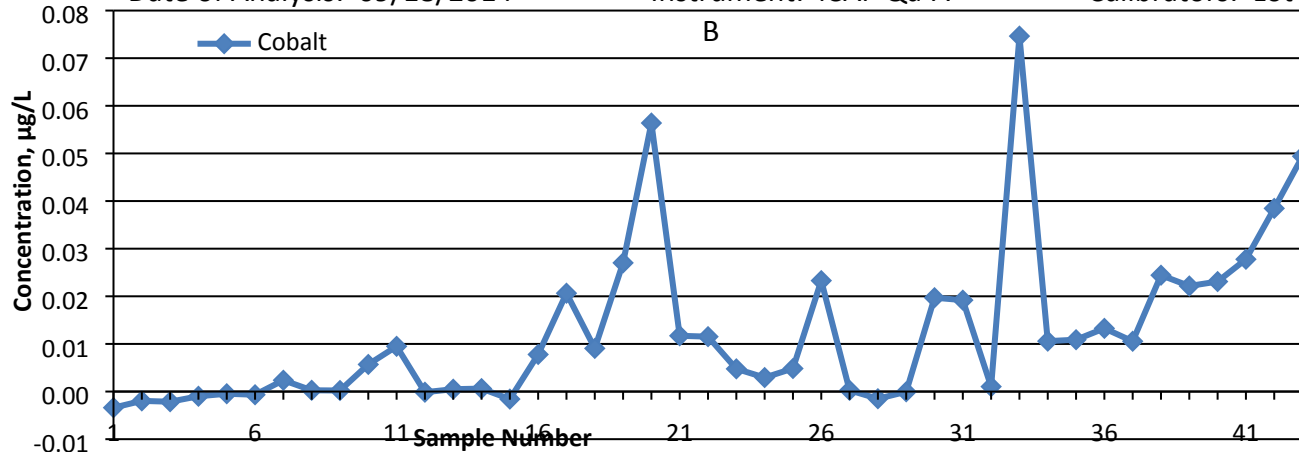


d. Cobalt Aqueous Blank Data (Concentrations)

Date of Analysis: 09/18/2014

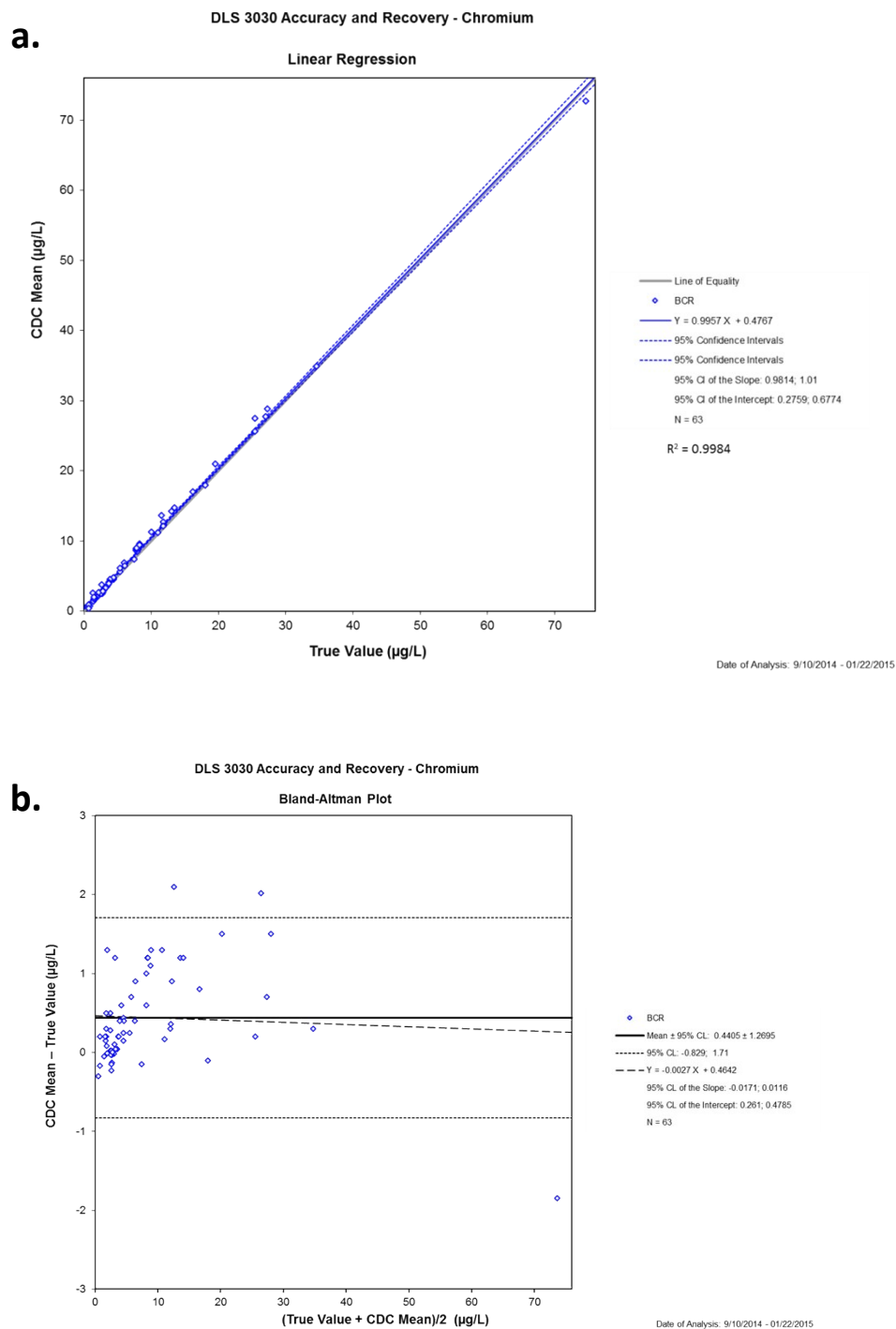
Instrument: iCAP Qa-A

Calibrators: Lot B

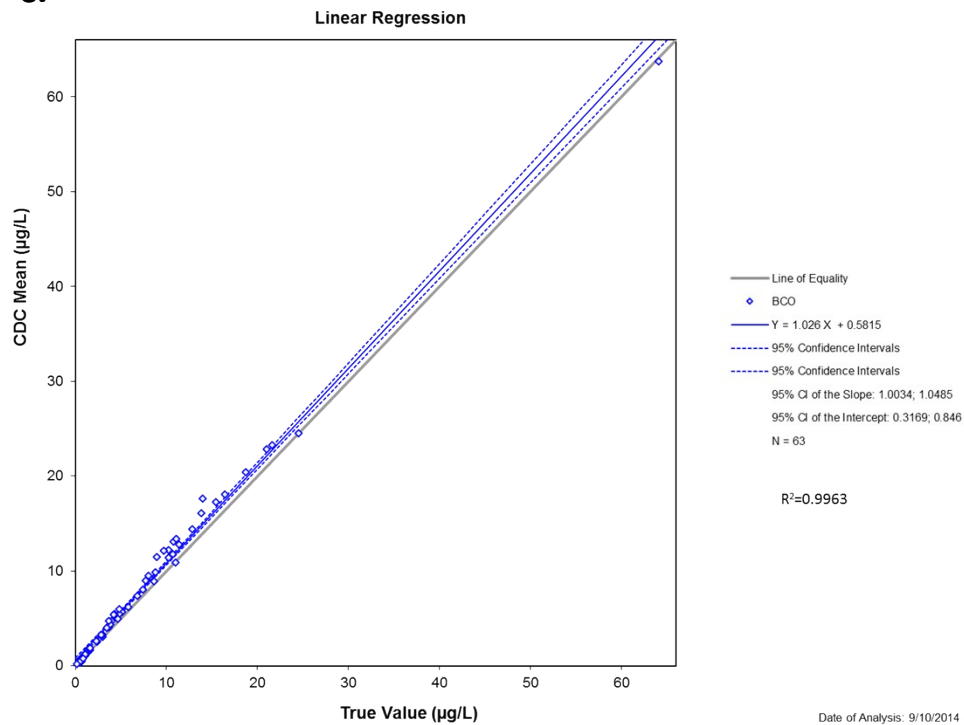


Accuracy

Figure S2a-d. Linear regression and Bland-Altman plots for Cr and Co for spiked materials and historical proficiency samples.



c. DLS 3030 Accuracy and Recovery - Cobalt



d. DLS 3030 Accuracy and Recovery - Cobalt

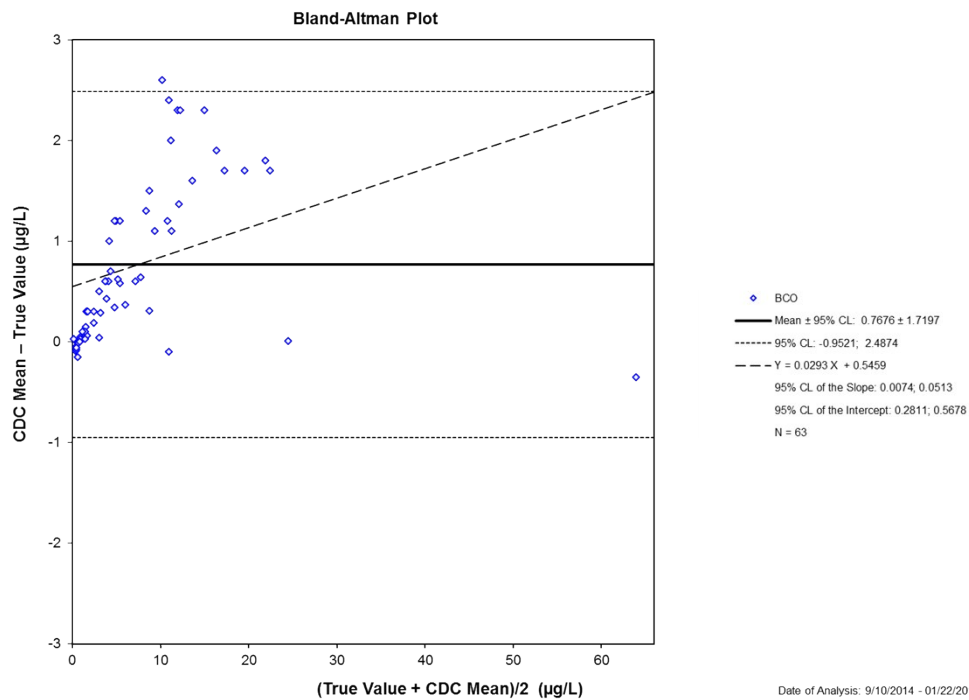


Table S2. New York Department of Health's (NYDOH) proficiency testing sample results for Cr.

NYDOH samples	True Value	CDC Overall AVG	CDC Overall SD	n (number of samples)	CDC Overall Bias	% Bias	CDC %CV	Recovery %
BE12-06	5.40	6.10	0.09	2	0.7	13	1.5	89
BE12-07	1.70	1.90	0.10	2	0.2	12	5.5	89
BE12-08	4.40	4.80	0.13	2	0.4	9	2.8	92
BE12-09	7.90	8.50	0.17	2	0.6	8	1.9	93
BE12-10	1.30	2.60	1.56	2	1.3	100	60.0	50
BE12-11	2.60	3.80	0.35	2	1.2	46	9.3	68
BE12-12	6.00	6.90	0.29	2	0.9	15	4.2	87
BE12-13	11.50	13.60	0.29	2	2.1	18	2.1	85
BE12-14	8.30	9.60	0.01	2	1.3	16	0.1	86
BE12-15	1.60	1.90	0.15	2	0.3	19	8.3	84
BE13-01	7.70	8.70	0.01	2	1.0	13	0.1	89
BE13-02	27.30	28.80	0.34	2	1.5	5	1.2	95
BE13-03	3.90	4.50	0.01	2	0.6	15	0.2	87
BE13-04	18.00	17.90	0.31	2	-0.1	-1	1.7	101
BE13-05	2.20	2.70	0.02	2	0.5	23	0.7	81
BE13-07	10.00	11.30	0.71	2	1.3	13	6.3	88
BE13-06	27.00	27.70	0.62	2	0.7	3	2.2	97
BE13-08	3.70	4.10	0.08	2	0.4	11	1.8	90
BE13-09	13.00	14.20	0.26	2	1.2	9	1.8	92
BE13-10	0.70	0.90	0.25	2	0.2	29	27.0	78
BE13-11	7.70	8.90	0.09	2	1.2	16	1.0	87
BE13-12	6.10	6.50	0.12	2	0.4	7	1.8	94
BE13-13	2.80	2.80	0.01	2	0.0	0	0.2	100
BE13-14	25.40	25.60	0.15	2	0.2	1	0.6	99
BE13-15	11.80	12.10	0.32	2	0.3	3	2.6	98
BE14-01	1.50	1.70	0.13	2	0.2	13	7.5	88
BE14-02	3.60	3.80	0.31	2	0.2	6	8.3	95
BE14-03	7.80	9.00	0.31	2	1.2	15	3.5	87
BE14-04	13.50	14.70	0.04	2	1.2	9	0.2	92
BE14-05	19.50	21.00	0.23	2	1.5	8	1.1	93
BE14-06	0.70	0.40	0.00	2	-0.3	-43	0.8	175
BE14-07	3.70	3.90	0.02	2	0.2	5	0.4	95
BE14-08	1.50	2.00	0.55	2	0.5	33	27.1	75
BE14-09	8.30	9.40	0.03	2	1.1	13	0.4	88
BE14-10	16.20	17.00	0.03	2	0.8	5	0.2	95

Table S3. New York Department of Health's (NYDOH) proficiency testing sample results for Co.

NYDOH samples	True Value	CDC Overall AVG	CDC Overall SD	n (number of samples)	CDC Overall Bias	% Bias	CDC %CV	Recovery %
BE12-06	13.80	16.10	0.12	2	2.3	17	1.5	86
BE12-07	10.80	13.10	0.10	2	2.3	21	5.5	82
BE12-08	4.00	4.70	0.01	2	0.7	18	2.8	85
BE12-09	2.30	2.60	0.01	2	0.3	13	1.9	88
BE12-10	8.00	9.50	0.02	2	1.5	19	59.9	84
BE12-11	3.50	4.10	0.08	2	0.6	17	9.3	85
BE12-12	3.80	4.40	0.01	2	0.6	16	4.2	86
BE12-13	11.10	13.40	0.15	2	2.3	21	2.1	83
BE12-14	10.20	12.20	0.06	2	2.0	20	0.1	84
BE12-15	1.40	1.50	0.00	2	0.1	7	8.3	93
BE13-01	9.70	12.10	0.01	2	2.4	25	0.1	80
BE13-02	21.60	23.30	0.02	2	1.7	8	1.2	93
BE13-03	4.30	5.50	0.04	2	1.2	28	0.2	78
BE13-04	14.00	17.60	0.13	2	3.6	26	1.7	80
BE13-05	1.40	1.70	0.02	2	0.3	21	0.7	82
BE13-07	4.80	6.00	0.04	2	1.2	25	6.3	80
BE13-06	16.40	18.10	0.03	2	1.7	10	2.2	91
BE13-08	4.20	5.40	0.03	2	1.2	29	1.8	78
BE13-09	8.90	11.50	0.01	2	2.6	29	1.8	77
BE13-10	1.10	1.20	0.02	2	0.1	9	27.0	92
BE13-11	2.80	3.30	0.00	2	0.5	18	1.0	85
BE13-12	8.80	9.90	0.05	2	1.1	13	1.8	89
BE13-13	18.70	20.40	0.00	2	1.7	9	0.2	92
BE13-14	10.70	11.80	0.10	2	1.1	10	0.6	91
BE13-15	6.80	7.40	0.05	2	0.6	9	2.6	92
BE14-01	3.40	4.00	0.03	2	0.6	18	7.5	85
BE14-02	1.50	1.80	0.07	2	0.3	20	8.3	83
BE14-03	7.70	9.00	0.04	2	1.3	17	3.5	86
BE14-04	12.80	14.40	0.06	2	1.6	13	0.2	89
BE14-05	15.40	17.30	0.07	2	1.9	12	1.1	89
BE14-06	0.80	0.80	0.03	2	0.0	0	0.8	100
BE14-07	3.70	4.70	0.04	2	1.0	27	0.4	79
BE14-08	1.60	1.90	0.02	2	0.3	19	27.1	84
BE14-09	10.20	11.40	0.12	2	1.2	12	0.4	89
BE14-10	21.00	22.80	0.06	2	1.8	9	0.2	92

Ruggedness

The results at the increased and decreased levels for each parameter were compared individually to the results obtained at the optimized parameters (20x dilution, 33 second rinse, 0.4% TMAH in the diluent, 5.0 mL/min He gas, and 1% ethanol in the diluent) for this method. The least squares means, 95% confidence intervals, and corresponding p-values are given.

When varying ethanol % and TMAH % in diluent, KED gas flow, and autosampler rinse time, QC concentration means fall within our QC acceptance range. The concentrations obtained when the dilution factor used during sample preparation was varied indicated that inaccurate values were obtained when a dilution factor other than 20X was used. A p-value < 0.05 indicated a statistically significant difference.

Table S4. Analytical results for Cr and Co in high QC analyzed as samples when the dilution factor used during sample preparation is varied

Chromium			
Sample IDs	15x dilution	20x dilution	25x dilution
	Concentration (µg/L)		
High QC-1A	17.2	11.7	11.2
High QC -1B	19.9	11.7	11.6
High QC -1C	18.4	11.6	10.7
High QC -1D	*	11.6	10.9
High QC -2A	18.3	11.6	11.6
High QC -2B	19.6	11.7	11.1
High QC -2C	19.1	11.6	9.99
High QC -2D	*	11.7	9.86
High QC -3A	17.8	11.6	9.86
High QC -3B	19.0	11.7	9.80
High QC -3C	17.2	11.8	10.4
High QC -3D	*	11.6	11.4
Least Squares Mean	18.5	11.7	10.7
Confidence Interval (95%)	(18.0, 19.0)	(11.2, 12.1)	(10.3, 11.1)
Comparison (decreased vs. method norm)	p<0.0001		
Comparison (increased vs. method norm)		p=0.001	
Cobalt			
Sample IDs	15x dilution	20x dilution	25x dilution
	Concentration (µg/L)		
High QC-1A	11.6	8.74	7.93
High QC -1B	13.2	8.51	8.25
High QC -1C	12.7	8.56	7.54
High QC -1D	*	8.59	7.74
High QC -2A	12.5	8.60	8.20
High QC -2B	13.7	8.56	7.93
High QC -2C	13.4	8.58	7.16
High QC -2D	*	8.48	6.99
High QC -3A	12.4	8.58	6.98
High QC -3B	13.3	8.56	7.04
High QC -3C	11.7	8.62	7.43
High QC -3D		8.54	8.00
Least Squares Mean	12.7	8.58	7.60
Confidence Interval (95%)	(12.4, 13.0)	(8.28, 8.88)	(7.30, 7.90)
Comparison (decreased vs. method norm)	p<0.0001		
Comparison (increased vs. method norm)		p<0.0001	

*For 15x dilution, there was not enough sample for aliquot "D".

Statistical Significance (p<0.05)

Table S5. Analytical results for Cr and Co in high QC analyzed as samples when the sample rinse time is varied

Chromium			
Sample IDs	24 s rinse	33 s rinse	39 s rinse
	Concentration (µg/L)		
High QC-1A	11.6	11.8	11.7
High QC -1B	11.5	12.0	12.4
High QC -1C	11.5	11.8	11.8
High QC -1D	11.5	11.9	11.8
High QC -2A	11.7	12.0	11.6
High QC -2B	11.4	11.8	11.9
High QC -2C	11.5	12.0	11.6
High QC -2D	11.8	11.8	11.7
High QC -3A	11.5	12.1	11.7
High QC -3B	11.5	11.9	11.6
High QC -3C	11.6	11.9	11.6
High QC -3D	12.6	11.8	11.4
Least Squares Mean	11.6	11.9	11.7
Confidence Interval (95%)	(11.5, 11.8)	(11.8, 12.0)	(11.6, 11.9)
Comparison (decreased vs. method norm)	p=0.0128		
Comparison (increased vs. method norm)		p=0.0817	
Cobalt			
Sample IDs	24 s rinse	33 s rinse	39 s rinse
	Concentration (µg/L)		
High QC-1A	8.68	8.60	8.68
High QC -1B	8.63	8.66	8.66
High QC -1C	8.63	8.60	8.65
High QC -1D	8.69	8.62	8.68
High QC -2A	8.82	8.73	8.73
High QC -2B	8.71	8.70	8.80
High QC -2C	8.76	8.67	8.72
High QC -2D	8.80	8.67	8.69
High QC -3A	8.73	8.79	8.75
High QC -3B	8.76	8.64	8.77
High QC -3C	8.76	8.65	8.70
High QC -3D	9.65	8.69	8.66
Least Squares Mean	8.80	8.67	8.71
Confidence Interval (95%)	(8.66, 8.94)	(8.53, 8.81)	(8.57, 8.85)
Comparison (decreased vs. method norm)	p=0.0441		
Comparison (increased vs. method norm)		p=0.5421	

Statistical Significance (p<0.05)

Table S6. Analytical results for Cr and Co in high QC analyzed as samples when the percentage of TMAH in the diluent is varied

Chromium			
Sample IDs	0.3% TMAH	0.4% TMAH	0.5% TMAH
	Concentration (µg/L)		
High QC-1A	11.9	12.7	12.5
High QC -1B	12.2	12.3	12.9
High QC -1C	12.2	12.3	12.5
High QC -1D	12.1	12.2	12.6
High QC -2A	11.9	12.6	12.6
High QC -2B	12.0	12.8	12.5
High QC -2C	12.0	12.2	12.4
High QC -2D	11.8	13.1	12.4
High QC -3A	12.0	12.2	12.4
High QC -3B	12.1	12.8	12.5
High QC -3C	11.8	12.4	12.9
High QC -3D	11.9	12.3	11.5
Least Squares Mean	12.0	12.5	12.5
Confidence Interval (95%)	(11.8, 12.2)	(12.3, 12.7)	(12.31, 12.66)
Comparison (decreased vs. method norm)	p=0.0002		
Comparison (increased vs. method norm)		p=0.9767	
Cobalt			
Sample IDs	0.3% TMAH	0.4% TMAH	0.5% TMAH
	Concentration (µg/L)		
High QC-1A	9.41	9.18	9.52
High QC -1B	9.46	9.14	9.48
High QC -1C	9.27	9.07	9.50
High QC -1D	9.26	9.01	9.48
High QC -2A	9.28	9.29	9.54
High QC -2B	9.39	9.29	9.53
High QC -2C	9.30	9.14	9.42
High QC -2D	9.31	8.81	9.51
High QC -3A	9.27	8.96	9.48
High QC -3B	9.21	9.12	9.51
High QC -3C	9.33	9.23	9.54
High QC -3D	9.33	9.23	8.23
Least Squares Mean	9.32	9.12	9.40
Confidence Interval (95%)	(9.18, 9.45)	(8.99, 9.26)	(9.26, 9.53)
Comparison (decreased vs. method norm)	p=0.0463		
Comparison (increased vs. method norm)		p=0.0069	

Statistical Significance (p<0.05)

Table S7. Analytical results for Cr and Co in high QC analyzed as samples when the percentage of ethanol used in the diluent is varied

Chromium			
Sample IDs	0.8% ethanol	1% ethanol	1.2% ethanol
	Concentration (µg/L)		
High QC-1A	11.4	12.0	11.8
High QC -1B	11.1	11.3	11.8
High QC -1C	11.3	11.3	11.8
High QC -1D	11.1	11.3	11.8
High QC -2A	11.2	11.2	11.7
High QC -2B	11.4	11.3	11.7
High QC -2C	11.1	11.2	11.7
High QC -2D	11.2	11.1	11.6
High QC -3A	11.2	11.2	11.8
High QC -3B	11.6	11.1	11.7
High QC -3C	11.5	11.2	11.8
High QC -3D	11.1	11.2	10.9
Least Squares Mean	11.3	11.3	11.7
Confidence Interval (95%)	(11.1 , 11.4)	(11.1, 11.4)	(11.5, 11.8)
Comparison (decreased vs. method norm)	p=0.7707		
Comparison (increased vs. method norm)		p<0.0001	
Cobalt			
Sample IDs	0.8% ethanol	1% ethanol	1.2% ethanol
	Concentration (µg/L)		
High QC-1A	8.38	8.57	8.40
High QC -1B	8.38	8.53	8.49
High QC -1C	8.53	8.58	8.42
High QC -1D	8.39	8.57	8.44
High QC -2A	8.54	8.61	8.52
High QC -2B	8.52	8.61	8.44
High QC -2C	8.48	8.52	8.48
High QC -2D	8.56	8.50	8.38
High QC -3A	8.58	8.63	8.45
High QC -3B	8.77	8.50	8.43
High QC -3C	8.50	8.52	8.42
High QC -3D	8.51	8.54	7.55
Least Squares Mean	8.51	8.56	8.37
Confidence Interval (95%)	(8.42 , 8.61)	(8.46, 8.65)	(8.27, 8.47)
Comparison (decreased vs. method norm)	p=0.5079		
Comparison (increased vs. method norm)		p=0.0084	

Statistical Significance (p<0.05)

Table S8. Analytical results for Cr and Co in high QC analyzed as samples when the KED He gas flow rate is varied

Chromium			
Sample IDs	4 mL/min	5 mL/min	6 mL/min
	Concentration (µg/L)		
High QC-1A	12.9	12.5	12.1
High QC -1B	13.0	12.5	12.1
High QC -1C	13.1	12.3	12.1
High QC -1D	13.0	12.2	12.0
High QC -2A	13.2	12.2	12.6
High QC -2B	13.2	12.3	12.2
High QC -2C	13.1	12.2	12.1
High QC -2D	13.0	12.1	11.9
High QC -3A	13.4	12.2	12.1
High QC -3B	12.8	12.2	11.9
High QC -3C	12.9	12.2	11.9
High QC -3D	12.9	12.1	12.0
Least Squares Mean	13.0	12.3	12.1
Confidence Interval (95%)	(12.9, 13.2)	(12.1, 12.4)	(11.9, 12.2)
Comparison (decreased vs. method norm)	p<0.0001		
Comparison (increased vs. method norm)		p=0.008	
Cobalt			
Sample IDs	4 mL/min	5 mL/min	6 mL/min
	Concentration (µg/L)		
High QC-1A	8.93	8.95	9.06
High QC -1B	8.90	9.01	9.09
High QC -1C	8.98	8.89	9.14
High QC -1D	8.96	8.92	9.03
High QC -2A	9.00	8.95	9.10
High QC -2B	9.11	9.00	9.06
High QC -2C	8.97	9.03	9.17
High QC -2D	8.95	9.02	9.11
High QC -3A	9.21	9.04	9.19
High QC -3B	8.91	9.01	9.10
High QC -3C	8.95	9.04	9.13
High QC -3D	8.95	8.99	9.17
Least Squares Mean	8.99	8.99	9.11
Confidence Interval (95%)	(8.92, 9.05)	(8.92, 9.06)	(9.04, 9.18)
Comparison (decreased vs. method norm)	p=0.9185		
Comparison (increased vs. method norm)		p<0.0001	

Statistical Significance (p<0.05)

Linearity

Table S9. Results of the analysis of calibration verification samples analyzed with the regular calibration curve (N=9 per each concentration)

Samples	Expected concentration Co and Cr, µg/L	Average measured concentration Cr, µg/L	Average measured concentration Co, µg/L	Average recovery Cr, %	Average recovery Co, %
1	20	21.4	22.7	107	113
2	50	51.2	53.1	102	106
3	100	108	111	108	111

Table S10. Calibration verification samples' target vales, measured concentrations and % recovery analyzed with extended calibration curve (N=6 per each concentration except samples between 150 and 750 µg/L where N=3)

Elevated Samples	Expected concentration µg/L	Average measured concentration Cr, µg/L	Average measured concentration Co, µg/L	Average recovery Cr, %	Average recovery Co, %
1	150	141	135	94	90
2	250	242	232	97	93
3	500	468	538	94	108
4	750	707	832	94	111
5	1000	946	1109	95	111
6	1500	1457	1633	97	109
7	2000	2306	2173	115	109
8	2500	2919	2760	117	110
9	3000	3418	3321	114	111
10	5000	5722	5562	114	111

Range

To quantify samples with concentrations falling above standard calibration curve ($>15\mu\text{g/L}$) we implemented the use of an extended calibration curve. Dilution of the samples is not used since % recoveries are outside the allowable range of 90-110%. To quantify samples with concentrations above those of the extended calibration curve ($> 100 \mu\text{g/L}$) we diluted samples with base blood. The maximum dilution factor is 50X with base blood for samples $\leq 5000 \mu\text{g/L}$. Dilution factors above 50X provide % recoveries outside of the acceptance range (90-110%).

Table S11. Average recoveries of the 25 and 100 $\mu\text{g/L}$ samples diluted with base blood

Dilution	Expected concentration, $\mu\text{g/L}$	Number of samples	Chromium			Cobalt		
			Average Concentration, $\mu\text{g/L}$	Standard Deviation	Average % Recovery	Average Concentration, $\mu\text{g/L}$	Standard Deviation	Average % Recovery
2x	25	3	25.9	0.6	104	24.8	0.9	99
5x	25	3	24.0	0.4	96	23.3	0.2	93
10x	100	5	122	22	122	123	14	123
20x	100	5	101	7.8	101	104	14	104

Table S12: Average recoveries of the high concentration blood samples diluted with deionized water.

Average Concentration (µg/L)	Expected Conc. (µg/L)	Average Recovery (%)	Standard deviation	RSD (%)	Dilution
Chromium					
1020	1000	102	1.5	1.4	10x
1036	1000	104	0.08	0.08	20x
5109	5000	102	1.6	1.6	50x
6264	6000	104	1.1	1.1	100x
7468	6500	115	0.3	0.21	100x
8223	7000	117	0.73	0.62	100x
10240	7500	137	4.2	3.1	100x
11324	10000	113	1.6	1.4	100x
24089	20000	120	2.8	2.4	200x
Cobalt					
935	1000	94	1.3	1.4	10x
949	1000	95	0.11	0.12	20x
4848	5000	97	1.2	1.2	50x
5584	6000	93	0.94	1.0	100x
6286	6500	97	0.18	0.19	100x
6978	7000	100	0.47	0.47	100x
7869	7500	105	1.5	1.4	100x
10329	10000	103	1.1	1.1	100x
21776	20000	109	3.1	2.9	200x

Table S13. Average recoveries of the high concentration blood samples diluted with base blood.

Average Concentration (µg/L)	Expected Conc. (µg/L)	Average Recovery (%)	Standard deviation	RSD (%)	Dilution
Chromium					
962	1000	96	3.9	4.0	20x
3254	3000	108	1.3	1.2	50x
4847	5000	97	6.8	7.0	100x
6125	5500	111	0.06	0.05	100x
6562	6000	109	6.9	6.3	100x
6361	7000	91	4.3	4.7	100x
10436	8000	130	2.1	1.6	100x
Cobalt					
979	1000	98	3.7	3.7	20x
3181	3000	106	1.5	1.5	50x
4758	5000	95	7.0	7.3	100x
6344	5500	115	0.62	0.54	100x
6875	6000	115	13.0	11	100x
6203	7000	88	5.2	5.9	100x
8724	8000	109	2.1	2.0	100x

Table S14. Average recoveries of the high concentration blood samples diluted with base blood 50x.

Average Concentration (µg/L)	Expected Conc. (µg/L)	Average Recovery (%)	Standard deviation	RSD (%)	Dilution
Chromium					
3062	3000	102	1.6	1.6	50x
4926	5000	99	1.3	1.3	50x
Cobalt					
3001	3000	100	2.0	2.0	50x
4853	5000	97	2.8	2.90	50x