Supplementary Materials: Comparison of Simultaneous Quantitative Analysis of Methylmercury and Inorganic Mercury in Cord Blood Using LC-ICP-MS and LC-CVAFS: The Pilot Study of the Japan Environment and Children's Study

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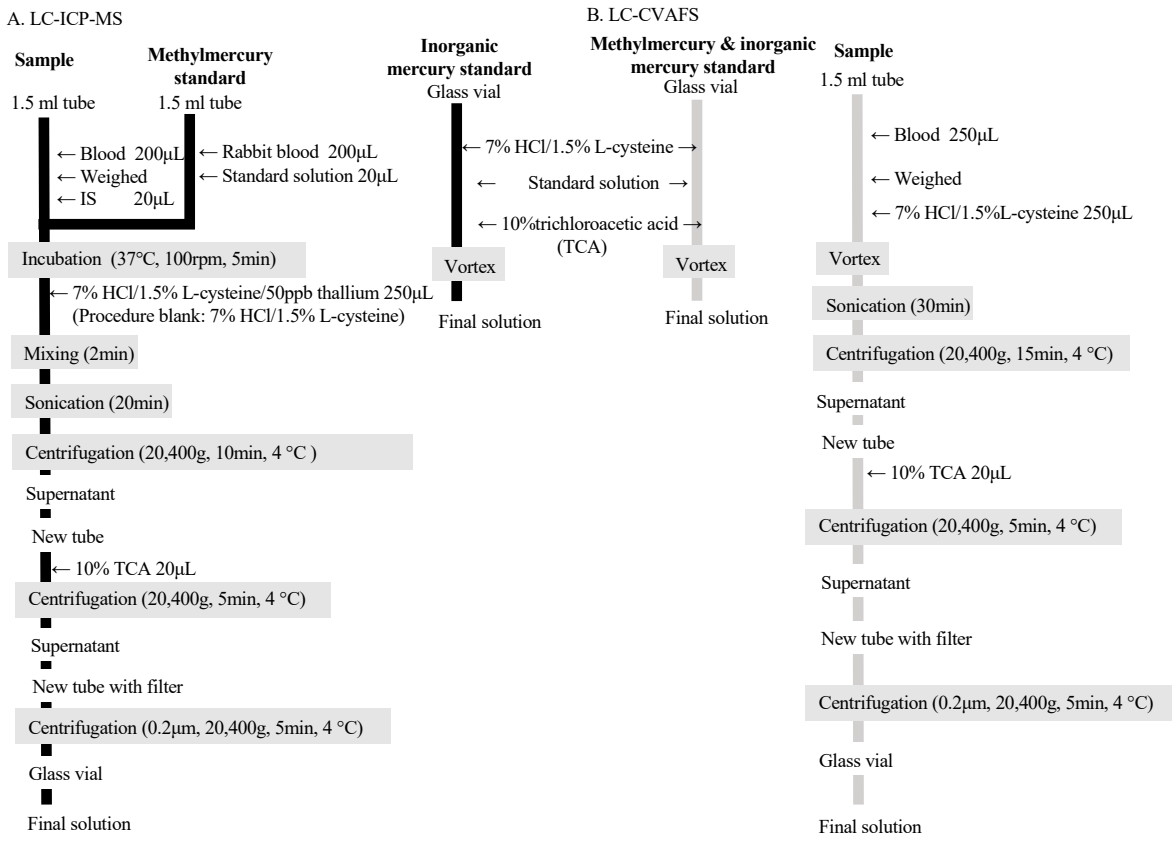

Figure S1. Pretreatment procedures. (**A**) and (**B**) showed the pretreatment procedures for LC-ICP-MS and LC-CVAFS, respectively.

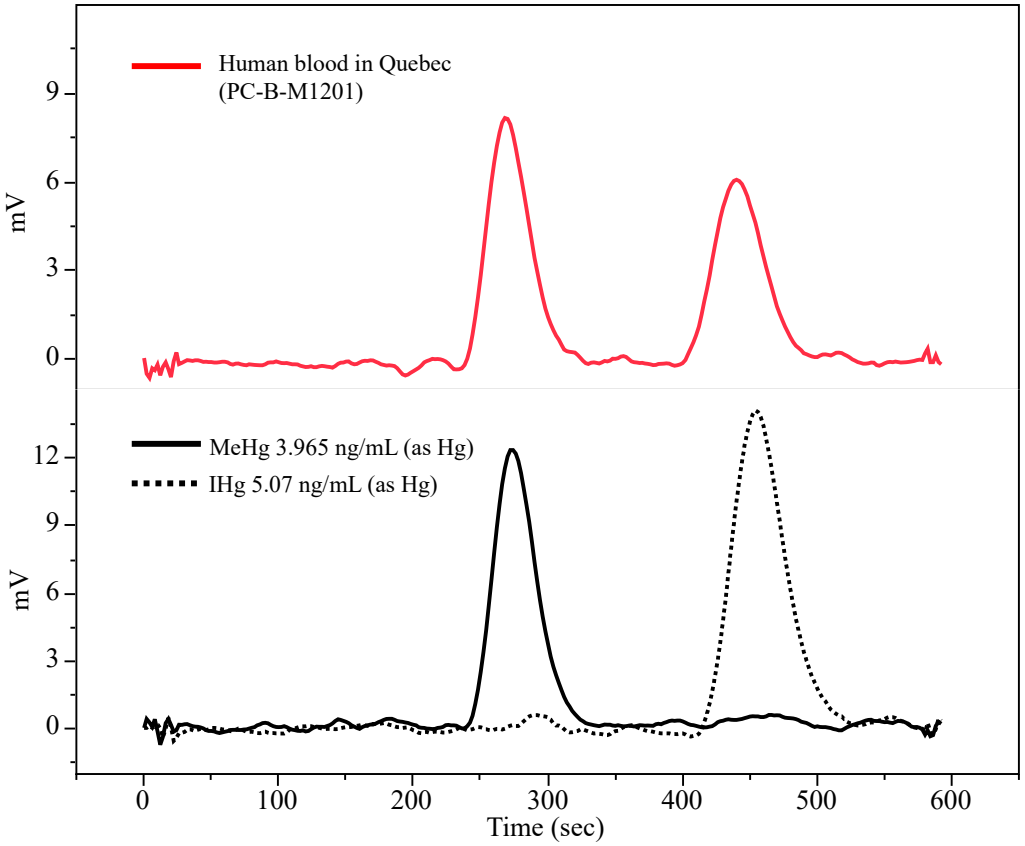

Figure S2. Chromatographic separation of standard solutions and blood containing methylmercury and inorganic mercury by LC-CVAFS. Upper, red line: human blood in Quebec (PC-B-M1201). Lower, black line: 3.965 ng/mL methylmercury (MeHg) standard solution (as Hg). Lower, dotted line: 5.07 ng/mL inorganic mercury (IHg) standard solution (as Hg).

Table S1. Calibration curve for mercury analysis.

Method		Calibration	
		Range (ng/mL)	r^2 (min-max)
LC-ICP-MS	Methylmercury	0.08–39.9	0.997–0.999
	Inorganic mercury	0.05–2.5	0.995–0.999
LC-CVAFS	Methylmercury	0.397–39.7	0.999–1.000
	Inorganic mercury	0.5–20.0	0.999–0.999

LC-ICP-MS: liquid chromatography-inductively coupled plasma mass spectrometry, LC-CVAFS: liquid chromatography-cold vapor atomic fluorescence spectrometry.

Table S2. Repeatability: repeated measurements of concentrations (ng/mL) in reference materials.

LC-ICP-MS		Target value (acceptable range)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Pooled blood	MeHg		8.84	9.29	9.58	9.35	9.11	9.72	9.04	9.75
	IHg		0.38	0.40	0.38	0.43	0.41	0.40	0.37	0.34
	THg		9.23*	9.69*	9.96*	9.78*	9.52*	10.12*	9.41*	10.09*
PC-B-M 1601	MeHg		8.61	9.47	9.21	9.92	8.74	9.85	9.44	9.80
	IHg		1.28	1.19	1.15	1.21	1.40	1.27	1.14	1.11
	THg	10.35 (7.96–12.74)	9.89*	10.7*	10.4*	11.1*	10.1*	11.1*	10.6*	10.9*

LC-CVAFS		Target value (acceptable range)	Run 1	Run 2	Run 3
Pooled blood	MeHg		9.96	8.87	9.54
	IHg		0.26	0.49	0.31
	THg		10.22*	9.36*	9.85*
Seronorm whole blood (Level 2)	MeHg	1.27 (0.76–1.77)	1.58	1.31	1.48
	IHg		14.91	14.09	14.90
	THg	17.0 (13.6–20.4)	16.49*	15.40*	16.38*
PC-B-M1201	MeHg		5.20	4.82	5.32
	IHg		4.65	4.64	4.56
	THg	9.47 (7.04–11.9)	9.85*	9.46*	9.88*
PC-B-M1203	MeHg		1.35	1.02	1.23
	IHg		0.78	1.09	0.95
	THg	2.37 (1.73–3.01)	2.13*	2.11*	2.18*

*the sum of methylmercury and inorganic mercury. LC-ICP-MS: liquid chromatography-inductively coupled plasma mass spectrometry, LC-CVAFS: liquid chromatography-cold vapor atomic fluorescence spectrometry, RSD: relative standard deviation. The trueness of the analytical method was assessed by analyzing four reference materials of human blood, which were Seronorm whole blood (level 2) and Qubec blood (PC-B-M1601, 1201, and 1203) purchased from Sero AS (Billingstad, Norway) and the Institute National de Santé Publique du Québec (Qubec, Canada). Human red blood cells and plasma for pooled blood were donated by the Japanese Red Cross Society (Tokyo, Japan). *the sum of methylmercury and inorganic mercury. LC-ICP-MS: liquid chromatography-inductively coupled plasma mass spectrometry, LC-CVAFS: liquid chromatography-cold vapor atomic fluorescence spectrometry, RSD: relative standard deviation. The trueness of the analytical method was assessed by analyzing four reference materials of human blood, which were Seronorm whole blood (level 2) and Qubec blood (PC-B-M1601, 1201, and 1203) purchased from Sero AS (Billingstad, Norway) and the Institute National de Santé Publique du Québec (Qubec, Canada). Human red blood cells and plasma for pooled blood were donated by the Japanese Red Cross Society (Tokyo, Japan).

Table S3. Accuracy of measured concentrations (ng/mL) in reference materials.

			Target value (acceptable range)	Mean (ng/mL)	SD (ng/mL)	Accuracy (%)
LC-ICP-MS	PC-B-M 1601	MeHg		9.38	0.50	
		IHg		1.21	0.09	
		THg	10.35 (7.96–12.74)	10.6	0.45	102
	PC-B-M1201	MeHg		5.47	0.07	
		IHg		4.99	0.09	
		THg	9.47 (7.04–11.9)	10.5	0.12	111
	PC-B-M1203	MeHg		1.32	0.04	
		IHg		1.14	0.03	
		THg	2.37 (1.73–3.01)	2.47	0.03	104
LC-CVAFS	Seronorm whole blood (Level 2)	MeHg	1.27 (0.76–1.77)	1.46	0.14	115
		IHg		14.6	0.47	
		THg	17.0 (13.6–20.4)	16.1	0.6	95
	PC-B-M1201	MeHg		5.11	0.26	
		IHg		4.62	0.05	
		THg	9.47 (7.04–11.9)	9.73	0.23	103
	PC-B-M1203	MeHg		1.20	0.17	
		IHg		0.94	0.15	
		THg	2.37 (1.73–3.01)	2.14	0.04	90

Table S4. Homogeneity results of metal concentrations in pooled blood.

Bottle no. - tube no.	Mn (ng/g)	Se (ng/g)	Cd (ng/g)	Hg (ng/g)	Pb (ng/g)
1-1	19.1	168	0.617	9.70	9.14
1-2	18.7	163	0.595	9.68	9.19
1-3	19.0	168	0.619	9.68	9.24
2-1	19.0	166	0.625	9.74	9.17
2-2	19.0	168	0.62	9.64	9.11
2-3	19.0	164	0.625	9.77	9.25
3-1	19.2	166	0.629	9.66	9.29
3-2	19.2	167	0.627	9.63	9.16
3-3	18.8	167	0.615	9.62	9.14
4-1	18.7	162	0.631	9.61	9.19
4-2	19.2	168	0.621	10.0	9.19
4-3	19.0	164	0.619	9.67	9.17
5-1	19.0	164	0.602	9.65	9.09
5-2	19.0	164	0.629	9.71	9.19
5-3	18.8	162	0.623	9.74	9.20
6-1	19.0	165	0.600	9.73	9.16
6-2	19.0	162	0.616	9.74	9.23
6-3	19.0	166	0.626	9.72	9.20
7-1	19.1	165	0.637	9.71	9.20
7-2	19.3	169	0.608	9.80	9.24
7-3	18.8	166	0.628	9.62	9.20
8-1	18.9	165	0.613	9.65	9.19
8-2	19.1	167	0.634	10.0	9.17
8-3	19.3	166	0.625	9.78	9.15
9-1	19.5	167	0.627	9.71	9.16
9-2	19.0	165	0.616	9.72	9.21
9-3	19.2	167	0.610	9.75	9.32
10-1	19.2	169	0.635	9.74	9.21
10-2	19.0	166	0.627	9.70	9.19
10-3	19.1	168	0.630	9.86	9.28
11-1	19.0	165	0.627	9.77	9.20
11-2	19.2	166	0.622	9.68	9.23
11-3	19.0	165	0.620	9.74	9.24
12-1	19.0	166	0.621	9.75	9.14
12-2	19.2	167	0.627	9.66	9.28
12-3	19.2	167	0.609	9.79	9.15
Mean	19.1	166	0.621	9.73	9.20
RSD (%) within bottles	0.80	0.94	1.46	0.73	0.53
RSD (%) between bottles	0.46	0.73	0.90	0.44	0.25
F value	0.743	1.41	0.957	0.640	0.527
P value	0.689	0.230	0.507	0.777	0.866

Mn: manganese, Se: selenium, Cd: cadmium, Hg: mercury, Pb: lead, RSD: relative standard deviation. Human red blood cells and plasma were donated by the Japanese Red Cross Society (Tokyo, Japan). Pooled blood samples were mixed, homogenized, and dispensed into ~2000 tubes in our laboratory. Homogeneity of the pooled blood was confirmed by analyzing five metals. We randomly selected 36 tubes. Metals were measured by ICP-MS, as described elsewhere (Nakayama et al. 2019). A one-way ANOVA revealed no significant differences between bottles.