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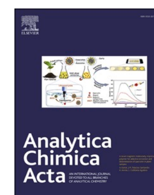

Method development and production of an ambient-stable blood certified reference material for total mercury, methylmercury, and trace elements

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HIGHLIGHTS

- A freeze-dried blood CRM was produced for THg, MeHg, and trace elements.
- The CRM remained stable during ambient-temperature transport and storage.
- Certified values were assigned from technically validated interlaboratory results across multi analytical platforms.
- The CRM supports global mercury monitoring frameworks under the Minamata Convention.

GRAPHICAL ABSTRACT

Frozen CRMs (-20 °C)

Ambient-stable (up to 60 °C)

Global QA/QC

Cold-chain required

Certified THg & MeHg

Effectiveness evaluation of the Minamata Convention

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ABSTRACT

Background: Certified reference materials (CRMs) for mercury speciation of biological fluids have hitherto been limited to frozen or cold-storage types, which impedes the participation of laboratories in tropical or remote regions. Existing CRMs often do not fully meet user needs, particularly regarding storage stability and concentration relevance.

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Blood
Stability
Minamata convention
QA/QC

Results: Using pooled Japanese human blood, we developed a freeze-dried, ambient-stable blood CRM, with certified values of 6.16 μg L^{-1} for total mercury (THg) and 5.46 μg L^{-1} for methylmercury (MeHg, as Hg). These concentrations are congruent with median exposure levels in fish-consuming populations (e.g., small island states and riverine Amazonian communities) derived from global biomonitoring data. Homogeneity, stability, and traceability were validated through inter-laboratory comparisons and rigorous uncertainty assessment.
Significance: This ambient-stable blood CRM broadens global access to high-quality QA/QC of mercury speciation, especially in regions lacking cold-chain infrastructure. It contributes to capacity building under the Minamata Convention and strengthens interlaboratory comparability. Registration in COMAR and international collaborative deployment are under active development, enhancing the global infrastructure for mercury biomonitoring.

1. Introduction

The Minamata Convention on Mercury provides a global legal framework for protecting human health and the environment from mercury emissions through robust analytical monitoring and evidence-based policies. Analytical chemistry plays a vital role in the implementation of the Convention, particularly in identifying contaminated sites (Article 12), protecting populations at risk of mercury exposure (Article 16), and evaluating the effectiveness of the Convention (Article 22). Reliable and comparable monitoring data are essential for these purposes, forming the scientific foundation of risk assessment and policy development.

Human biomonitoring (HBM) has been recognized as a key indicator for evaluating the effectiveness of the Convention, as adopted at the fourth and fifth meetings of the Conference of the Parties (COP-4 in Bali and COP-5 in Geneva). At these meetings, Parties advanced the first effectiveness evaluation cycle and adopted a set of indicators to assess progress under the Convention. Within this framework, HBM was identified as a primary line of evidence for tracking trends in mercury exposure. The Guidance on Monitoring of Mercury and Mercury Compounds to Support Evaluation of the Effectiveness of the Minamata Convention [1] proposes a tiered approach in which mercury concentrations in blood, urine, and hair serve as core indicators depending on the target population. The guidance further emphasizes the importance of quality assurance and quality control (QA/QC) in biomonitoring programs. In particular, the routine use of certified reference materials (CRMs) and participation in interlaboratory comparison (ILC) programs and/or proficiency testing (PT) are recommended to ensure analytical comparability and traceability of measurement results across laboratories. The World Health Organization (WHO) also provides detailed standard operating procedures (SOPs) for mercury measurement in biological matrices in its publication Assessment of Prenatal Exposure to Mercury [2], which recommends standardized blood mercury analysis for national biomonitoring programs. These frameworks highlight the importance of well-characterized certified reference materials to ensure comparability and traceability of biomonitoring data across laboratories.

However, existing CRMs for mercury in blood remain limited in number and format. Widely used materials such as NIST SRM 955d and Seronorm™ whole blood are supplied as frozen or refrigerated products, requiring cold-chain transport and storage. Freeze-dried blood reference materials such as ERM-DA634, ERM-DA635 and ERM-DA636 (European Commission, JRC) have recently been developed to improve transport stability under ambient conditions and provide certified values for several trace elements including total mercury [3]. However, these materials provide certified values only for total elemental concentrations and do not include mercury speciation such as methylmercury. Consequently, the availability of ambient-stable CRMs suitable for quality assurance of mercury speciation in blood remains limited, particularly for materials with concentrations relevant to biomonitoring in populations with higher seafood consumption.

Despite these advances, practical challenges remain for laboratories in tropical and low-resource regions, where reliable cold-chain infrastructure and access to certified reference materials are often limited. In addition, some existing materials contain mercury at concentrations representative of low-fish-consumption populations, which may be less suitable for evaluating laboratories analyzing samples from highly exposed populations. Moreover, information on the stability of blood CRMs at elevated transport temperatures (e.g., up to 60 °C) remains limited, despite its relevance for shipment to tropical regions.

Recent reviews have also highlighted the increasing global demand for reliable CRMs and harmonized analytical infrastructure following the Minamata Convention's entry into force [4]. Despite the treaty's adoption by more than 120 countries, anthropogenic mercury emissions remain high—particularly in Latin America, Africa, and Southeast Asia—where analytical capacity and metrological traceability are still developing. Therefore, the development of ambient-stable blood certified reference materials capable of supporting reliable mercury biomonitoring without cold-chain logistics remains an important analytical requirement for the global implementation of the Minamata Convention. In particular, information on CRM stability at elevated temperatures during transport (e.g., up to 60 °C) remains limited, despite its relevance for shipment to tropical regions.

Recent advances such as the dried blood spot (DBS) method have demonstrated the feasibility of mercury determination without cold storage, highlighting the potential of ambient-stable materials for biomonitoring. Santa-Ríos et al. [5] reported that methylmercury (MeHg) in DBS remains stable for extended periods at room temperature and correlates well with venous blood concentrations. These developments highlight the growing interest in ambient-stable sampling approaches and emphasize the need for well-characterized reference materials to support such analytical techniques.

In parallel with DBS-based approaches, volumetric absorptive microsampling (VAMS) has gained increasing attention as another non–cold-chain sampling method for trace elements. VAMS enables accurate collection of fixed blood volumes and has recently been shown to maintain stable concentrations of several metals—including mercury—for multiple months at room temperature and even at 37 °C, thereby demonstrating strong potential for biomonitoring in tropical climates and resource-constrained settings [6]. These advances reflect a broader trend toward ambient-stable sampling and reference materials.

This study aimed to develop and characterize an ambient-stable whole-blood CRM (NIMD-04) containing total mercury (THg), methylmercury (MeHg), and multiple trace elements. The material is designed to support quality assurance in laboratories lacking cold-chain infrastructure and to improve international comparability in human biomonitoring studies conducted under the Minamata Convention.

2. Materials and methods

2.1. Overview of the development process

The overall workflow for developing the blood CRM involved the optimization of preparation conditions, large-scale production under controlled conditions, and evaluation of homogeneity, stability, and characterization in accordance with ISO Guide 35:2017 [7], recently

updated as ISO 33405:2024 and ISO 17034:2016 [8]. All analytical steps were designed to ensure traceability, reproducibility, and biosafety of the resulting material.

2.2. Optimization of preparation conditions

Preparation parameters were optimized to ensure both chemical stability of mercury species and physical homogeneity of the blood matrix. Commercially available human whole blood (Asian origin, pre-screened for infectious markers) was used to test the following preparation parameters: concentration (1.8 mg g^{-1} and 3.6 mg g^{-1}) of the anticoagulant dipotassium ethylenediaminetetraacetate (EDTA-2K), freeze-drying program, and gamma-ray sterilization. Three freeze-drying programs were tested, all of which shared the same pre-freezing (−40 °C, 40 min) and vacuum (−40 °C, 20 min) steps but differed in the secondary drying temperature and duration: Program 1 (−10 °C → 20 °C for 5 h), Program 2 (−10 °C → 10 °C for 10 h), and Program 3 (−10 °C → 5 °C for 20 h). Sterilization was examined on the basis of previously reported dose ranges (10–50 kGy) and performed at 21 kGy (7 kGy h^{-1} × 3 h) using a^{60}Co source.

After reconstitution with 3 mL of ultrapure water, THg and MeHg concentrations were measured to evaluate recovery and reproducibility. Lyophilization resulted in an average water loss of approximately 2.38 g per vial. For routine analytical use, reconstitution was standardized to 3.0 mL of ultrapure water to provide a simple and reproducible procedure for users. Because this volume does not exactly correspond to the original water content prior to freeze-drying, analytical results were evaluated based on the measured concentrations after standardized reconstitution.

To examine the effect of gamma-ray sterilization on mercury stability, quintuplicate vials containing EDTA-2K at the concentration of 1.8 or 3.6 mg g^{-1} were irradiated at 21 kGy using a^{60}Co gamma source. Non-irradiated samples containing 3.6 mg g^{-1} EDTA-2K were used as the control (0 kGy). After irradiation, THg and MeHg were analyzed on a mass basis to determine potential changes in the mercury content.

The higher concentration of EDTA-2K (3.6 mg g^{-1}) and the mildest drying program (Program 1; −10 °C/60 min → 20 °C/300 min) were selected because they prevented coagulation, ensured homogeneity, and maintained analyte stability. Gamma sterilization at 21 kGy showed no significant effect on THg or MeHg concentrations, confirming its suitability as the standard sterilization condition.

For short-term stability testing, gamma-sterilized, freeze-dried samples (3.6 mg g^{-1} EDTA-2K) were stored at 4, 20, and 37 °C for up to 4 weeks to simulate transport and storage environments. At 0, 1, 2, 3, and 4 weeks, one vial from each temperature condition was reconstituted and analyzed for THg and MeHg. No time-dependent degradation was observed at any temperature, demonstrating excellent short-term stability of the sterilized material.

2.3. Ethics and volunteer sampling

All human samples were collected and handled in accordance with approved ethical guidelines. The study protocol was reviewed and approved by the Ethics Committee for Epidemiological Research of the National Institute for Minamata Disease (Approval No. Epidemiology/21/002; 25 November 2021). Sixty healthy adults from Shizuoka Prefecture, Japan, volunteered to provide venous blood samples. Licensed medical staff collected seven samples in 7 mL EDTA-2K tubes (for CRM preparation) and one sample in 9 mL serum tube per donor. Serum was stored at 4 °C until serological testing, while blood for CRM preparation was stored at −80 °C and anonymized before processing.

2.4. Bulk preparation, dispensing, and lyophilization

Large-scale preparation of the candidate CRM (NIMD-04) was carried out to produce uniform, sterile, and stable blood materials. The preparation procedure consisted of blood collection with ethical approval, screening for infectious markers, pooling and mixing of screened blood, dispensing into vials with EDTA-2K anticoagulant, freeze-drying, and gamma sterilization.

All serum samples tested negative for major viral markers (HBsAg, HIV p24, HIV-1/2 antibodies, HCV antigen). The pooled blood was sterilized by gamma irradiation at a total dose of 21 kGy (7 kGy h^{-1} × 3 h) using a^{60}Co source, pooled in a 5 L borosilicate beaker, adjusted to a final EDTA-2K concentration of 3.6 mg g^{-1} (accounting for EDTA in collection tubes), gently stirred, and dispensed into 800 borosilicate vials (10 mL, inner-cap type), each of which was ≈3.00 g (filling error ±0.1%). Lyophilization used the optimized program: pre-freezing at −40 °C for 40 min, primary drying at−10 °C for 60 min (vacuum; condenser −40 °C), and secondary drying at 20 °C for 300 min. For testing, samples in vials were reconstituted with 3.0 mL of ultrapure water and gently shaken (∼20 strokes min^{-1}); complete reconstitution occurred within 3–6 h.

2.5. Homogeneity testing

The homogeneity of the prepared material was evaluated in accordance with ISO Guide 35:2017 [7]. Ten vials were selected across the production sequence, and two subsamples per vial were analyzed for THg, MeHg, and seven trace elements (Pb, Cd, Mn, Se, As, Cu, Zn) using the analytical procedures described in Section 2.7. One-way analysis of variance (ANOVA) was applied to estimate the between-bottle (S_bb) and the within-bottle (Sr) components. When ANOVA yielded a negative estimates of S_bb^2, the value was replaced with the corresponding U_bb^2 in accordance with ISO Guide 35:2017 [7].

For all certified analytes, the estimated between-bottle component was small enough that no correction for inhomogeneity was required for value assignment. The repeatability of subsamples, expressed as RSD (Sr), was consistently low across analytes, indicating good subsample repeatability rather than bottle-level homogeneity. Taken together, these results indicate that the material exhibits sufficient bottle-to-bottle consistency for the certification of THg and MeHg.

2.6. Stability evaluation

The stability of the blood CRM was assessed through both short-term (transport simulation) and long-term (storage) tests using an isochronous design.

2.6.1. Short-term stability (STS)

Short-term stability was evaluated at 20, 37, and 60 °C to simulate shipping conditions. Samples were stored for 0, 1, 2, 3, and 4 weeks, and all vials were analyzed simultaneously. Linear regression was applied to the plot of concentration (Y) versus time (X) to test for significant trends (two-tailed t, 95% CL). The short-term stability uncertainty component (u_sts) was calculated as $s(b_1) \times X$ (Table 1 summarizes results).

2.6.2. Long-term stability (LTS)

Long-term stability was tested over 12 months at −20 °C and −80 °C to assess storage stability. Samples transferred at 12, 9, 6, 3, and 0 months were analyzed together at the end of the study. Regression analysis was performed as in the same manner as for the short-term stability test, and the LTS uncertainty (u_lts) was calculated as $s(b_1) \times X$.

2.7. Analytical methods

Analytical homogeneity and stability tests were conducted to ensure consistency and traceability throughout the development of the candidate CRM.

Total mercury (THg): Cold vapor atomic absorption spectrometry (CV-AAS; Hg-201, Sanso Seisakusho, Tokyo, Japan) was performed after wet digestion with mixed acids (HNO_3–$HClO_4$–H_2SO_4, 220 °C). Tin(II)

Table 1
Summary of results from short-term stability testing of all elements in the blood CRM.

Element	Temperature (°C)	Regression slope (b_1)	$s(b_1)$	Significant trend	Relative stability uncertainty (4 weeks, %)
THg	20	−0.01	0.06	No	3.6
THg	37	−0.07	0.03	No	1.7
THg	60	0.01	0.02	No	1.1
MeHg	20	0.02	0.02	No	1.2
MeHg	37	0.01	0.03	No	1.9
MeHg	60	−0.04	0.03	No	2.0
Pb	20	0.09	0.04	No	2.4
Pb	37	0.03	0.04	No	2.2
Pb	60	0.04	0.01	No	0.8
Cd	20	0.01	0.00	No	2.1
Cd	37	0.01	0.01	No	2.5
Cd	60	0.01	0.00	No	1.4
Mn	20	0.10	0.40	No	8.5
Mn	37	1.4	0.70	No	15.6
Mn	60	−0.3	0.50	No	9.0
Se	20	2.0	2.0	No	4.2
Se	37	1.0	1.0	No	2.0
Se	60	1.0	1.00	No	1.7
As	20	0.05	0.03	No	4.1
As	37	0.02	0.01	No	1.8
As	60	0.04	0.03	No	3.6
Cu	20	13	10	No	5.0
Cu	37	2.0	5.0	No	2.5
Cu	60	5.7	2.0	No	0.9
Zn	20	0.07	0.05	No	4.1
Zn	37	0.00	0.03	No	2.4
Zn	60	0.03	0.02	No	1.2

chloride (1%, w/v) was used as the reducing agent following the Mercury Analysis Manual of the Japanese Ministry of the Environment [9].

Methylmercury (MeHg): Gas chromatography with electron capture detection (GC-ECD; G2700, Yanaco Keisoku, Kyoto, Japan) was performed after alkaline digestion (KOH–ethanol), extraction with dithizone–toluene, back-extraction with Na_2S, and re-extraction with dithizone–toluene, as described in the Mercury Analysis Manual [9].

Multi-element analysis (Cu, Zn, Se, As, Pb, Cd, Mn): Inductively coupled plasma quadrupole mass spectrometry (ICP-QMS; 7700x, Agilent Technologies, Santa Clara, CA, USA) was performed in accordance with the JECS procedure [10]. Participating laboratories employed their own validated methods based on these principles; detailed instrumentation and analytical conditions are summarized in Tables 2 and 3.

2.8. Characterization and value assignment

Interlaboratory characterization was conducted to assign certified values and establish metrological traceability of the CRM. Fourteen participating laboratories from five countries were involved in the study. These included ISO/IEC 17025-accredited analytical laboratories, research laboratories with extensive experience in mercury speciation analysis, and instrument manufacturers involved in the development and validation of analytical systems for mercury determination. Laboratories holding ISO/IEC 17025 accreditation routinely perform trace element analysis under accredited quality management systems. For research laboratories without ISO/IEC 17025 accreditation, competence was assessed based on their previous participation in mercury-related proficiency testing programs and involvement in certified reference material characterization studies. Instrument manufacturers participated to evaluate the performance of analytical systems for mercury determination in biological matrices; however, their analytical results were treated in the same manner as those from other participating laboratories during the statistical evaluation.

Prior to the interlaboratory comparison, participating laboratories provided information on their analytical procedures and reported limits

Table 2
Analytical methods used by participating laboratories for analyzing total mercury (THg) and methylmercury (MeHg) in the blood CRM (NIMD-04). The column "No. of satisfactory laboratory results" indicates the number of laboratory results that fulfilled the predefined analytical performance criteria described in Section 2.8. Results not meeting these criteria (e.g., repeatability, quality-control agreement, spike recovery, or blank requirements) were excluded from value assignment. Abbreviations: CV-AAS, cold-vapor atomic absorption spectrometry; CV-AFS, cold-vapor atomic fluorescence spectrometry; TDA-AAS, thermal decomposition amalgamation AAS; ICP-QMS, inductively coupled plasma quadrupole mass spectrometry; GC-CVAFS, gas chromatography–cold-vapor atomic fluorescence spectrometry; HPLC-ICP-MS, high-performance liquid chromatography coupled to ICP-MS; HPLC-CL, high-performance liquid chromatography with chemiluminescence detection; GC-ECD, gas chromatography–electron capture detector.

Element	Instrumental analysis	Sample preparation	No. of laboratories	No. of satisfactory results
THg	CV-AAS [9]	H_2SO_4–HNO_3–$HClO_4$; open system	2	2
	CV-AFS [11]	HNO_3; microwave digestion	1	1
	TDA-AAS (direct combustion) [12]	No reagents; direct analysis by thermal decomposition AAS; EPA Method 7473	4	4
	TDA-AAS (alkaline digestion) [13]	NaOH or mild pretreatment before thermal decomposition AAS	2	2
	ICP-QMS [6,11,14,15]	HNO_3–HCl or alkaline dilution with EDTA, TMAH, Triton-X, 1-BuOH	4	3
MeHg	GC-CVAFS [11]	Acid digestion; ethylation; GC separation	2	1
	HPLC-ICP-MS [14,15]	Alkaline (TMAH), sonication, ultrafiltration; C18-column separation	2	2
	HPLC-CL [16,17]	Alkaline digestion; solvent extraction; HPLC-chemiluminescence detection	1	1
	TDA-AAS [13]	Solvent extraction followed by thermal decomposition measurement	1	1
	GC-ECD [9]	Alkaline (KOH/ethanol) digestion; dithizone–toluene extraction; Na_2S back-extraction	3	2

of quantification (LOQ) for total mercury (THg) and methylmercury (MeHg). Each laboratory received 3–5 vials of NIMD-04 together with a commercially available Seronorm® Trace Elements Whole Blood (Lot 20119 3333) quality control material. This material is a routine quality-control material rather than a certified reference material and was analyzed alongside the study samples as an auxiliary check of laboratory analytical performance.

For THg and MeHg, laboratories used cold-vapor atomic absorption or fluorescence spectrometry (CV-AAS, CV-AFS) [9,11], thermal decomposition amalgamation AAS (TDA-AAS) [12,13], inductively coupled plasma quadrupole mass spectrometry (ICP-QMS), and chromatographic techniques coupled with CV-AFS [11], ICP-QMS [6,10,14,15], electron capture detector (ECD) [9], or chemiluminescence (CL) [16,17]. For THg determination by CV-AAS or CV-AFS, blood samples were typically subjected to acid digestion prior to reduction of Hg^{2+} to elemental mercury (Hg^0) and vapor generation for detection. Some laboratories also applied direct thermal decomposition (TDA-AAS)

Table 3
Sample preparation methods used by participating laboratories for analyzing Pb, Cd, Mn, Se, As, Cu, and Zn in the blood CRM (NIMD-04) by ICP-QMS. Results are expressed as "No. of satisfactory results (results meeting the predefined performance criteria described in Section 2.8/total laboratories."

Sample preparation type	Reagents and procedure	Pb	Cd	Mn	Se	As	Cu	Zn
Acid digestion	HNO_3 or HNO_3–HCl; microwave or open-system digestion	2/2	2/2	3/3	3/3	2/3	3/3	3/3
Alkaline dilution	EDTA–TMAH–Triton-X–1-BuOH solution	3/3	3/3	2/3	3/3	3/3	3/3	3/3
Acidified dilution with stabilizer	0.5 % HNO_3+ 0.05 % Triton-X + 2 % methanol + 0.25 mg L^{-1} Au; no digestion	0/1	1/1	1/1	1/1	1/1	0/1	1/1
Ammonium hydroxide dilution	NH_4OH only; no acid digestion	0/1	0/1	0/1	1/1	0/1	1/1	0/1

without chemical digestion. For MeHg analysis, laboratories applied solvent extraction or alkaline digestion followed by derivatization and chromatographic separation before detection. For multi-element determination (Pb, Cd, Mn, Se, As, Cu, Zn), ICP-QMS was employed following acid or alkaline digestion procedures. The analytical methods and sample-preparation procedures used by the participating laboratories are summarized in Table 2 (for THg and MeHg) and Table 3 (for other trace elements).

To ensure analytical validity, predefined performance criteria were applied to the reported data prior to statistical evaluation. These criteria included (A) repeatability of replicate analyses of NIMD-04 within each laboratory (RSD $\leq$10%, n = 8 per laboratory); (B) agreement of the Seronorm® control result with the manufacturer-provided target value within the stated acceptable range; (C) spike recovery at concentration levels close to the sample concentration (90–110%); and (D) acceptable blank levels, defined as 10 *s* of blank measurements not exceeding the measured value. Results that did not meet these predefined criteria were excluded before statistical analysis. The overall procedure for accepting laboratory results and assigning certified values is summarized in Fig. 1. The remaining datasets were then evaluated for potential statistical outliers using Grubbs' test (α = 0.05). Certified values were calculated as the mean of the technically valid laboratory results.

Metrological traceability of the assigned values was ensured through calibration with commercially available standard solutions traceable to international reference standards. The evaluation of certified values and their associated uncertainties followed the general principles described in ISO Guide 35:2017.

2.9. *Uncertainty estimation and combination*

The uncertainty budget for each analyte was constructed following ISO Guide 98-3 (GUM) to quantify all significant contributors to the measurement uncertainty. The combined standard uncertainty (u_c) was obtained from characterization, homogeneity, and stability components:

$$u_c^2 = u_char^2 + u_bb^2 + u_lts^2 + u_sts^2,$$

Where u_c is the combined standard uncertainty, representing the uncertainty associated with the certified value; u_char is the uncertainty due to characterization, derived from the interlaboratory comparison used to assign the certified value and reflecting the variability among participating laboratories; u_bb is the uncertainty due to between-bottle heterogeneity, accounting for possible differences among individual units of the CRM; u_lts is the uncertainty due to long-term stability, representing changes during prolonged storage; and u_sts is the uncertainty due to short-term stability, representing potential variation during transportation. Expanded uncertainties (k = 2) were used for certified values reported in volume concentration. Table 4 lists all uncertainty components and their relative contributions.

3. Results

3.1. *Optimization of preparation and preservation conditions*

Optimization experiments confirmed that the selected conditions (EDTA-2K concentration of 3.6 mg g^{-1}, freeze-drying program 1, and

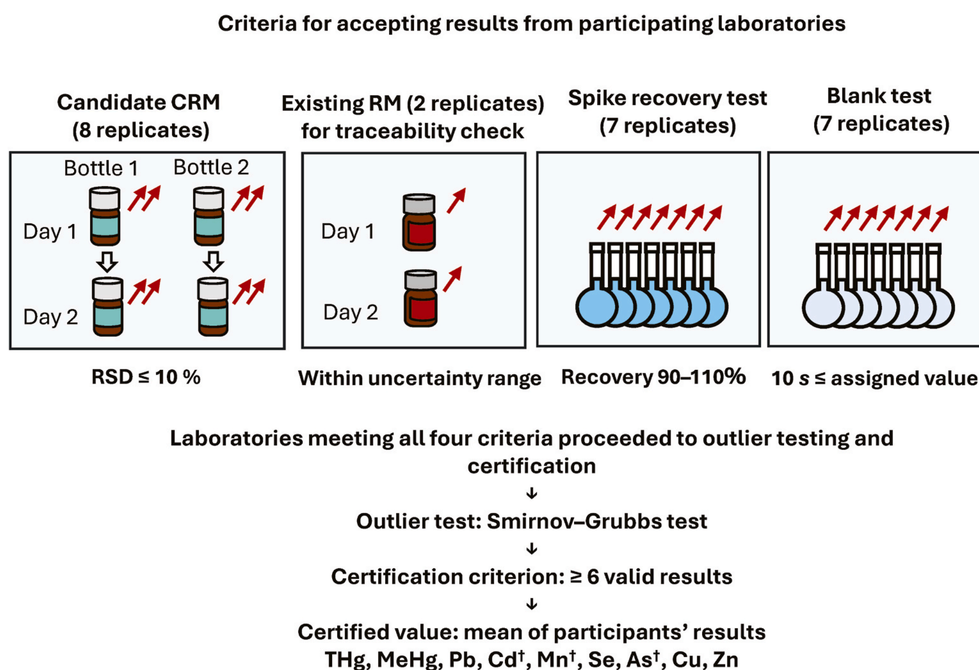

Fig. 1. Procedure for assigning certified values using the results from participating laboratories. Acceptance criteria were RSD $\leq$10%, within uncertainty range, recovery 90%–110%, and 10 *s* $\leq$ candidate value. Laboratories satisfying all four criteria proceeded to outlier testing (Smirnov–Grubbs test) and certification according to ISO Guide 35:2017. †Cd, Mn, and As are provided as indicative values (non-certified).

Table 4
Summary of uncertainty components and combined uncertainty for characterizing the blood CRM (NIMD-04).

Element	Assigned value	u_char	u_bb	u_lts	u_sts	u_c	U (k = 2)	Certification
THg	6.16 μg L^{-1}	0.14 (2.3 %)	0.15 (2.5 %)	0.04 (0.7 %)	0.23 (3.7 %)	0.31 (5.0 %)	0.62 (10 %)	Certified
MeHg as Hg	5.46 μg L^{-1}	0.18 (3.3 %)	0.05 (1.0 %)	0.11 (2.0 %)	0.13 (2.3 %)	0.25 (4.6 %)	0.50 (9.2 %)	Certified
Pb	6.76 μg L^{-1}	0.11 (1.6 %)	0.10 (1.4 %)	0.20 (3.0 %)	0.17 (2.5 %)	0.30 (4.4 %)	0.60 (8.9 %)	Certified
Cd	0.881 μg L^{-1}	0.054 (6.1 %)	0.023 (2.6 %)	0.026 (2.9 %)	0.021 (2.4 %)	0.067 (7.6 %)	0.134 (15.2 %)	Indicative
Mn	17.7 μg L^{-1}	0.8 (4.8 %)	2.3 (13.1 %)	2.1 (12.0 %)	2.7 (15.4 %)	4.2 (24.0 %)	8.5 (48.0 %)	Indicative
Se	0.182 mg L^{-1}	0.010 (5.4 %)	0.005 (2.5 %)	0.005 (2.5 %)	0.007 (4.0 %)	0.014 (7.6 %)	0.028 (15.3 %)	Certified
As	3.39 μg L^{-1}	0.24 (7.0 %)	0.06 (1.7 %)	0.10 (2.9 %)	0.13 (3.8 %)	0.29 (8.6 %)	0.58 (17.3 %)	Indicative
Cu	0.667 mg L^{-1}	0.017 (2.6 %)	0.016 (2.3 %)	0.018 (2.7 %)	0.038 (5.7 %)	0.045 (6.7 %)	0.090 (13.5 %)	Certified
Zn	4.82 mg L^{-1}	0.10 (2.0 %)	0.10 (2.1 %)	0.10 (2.0 %)	0.22 (4.5 %)	0.28 (5.7 %)	0.55 (11.5 %)	Certified

gamma irradiation at 21 kGy) ensured both chemical stability and reconstitution performance of the blood matrix. Comprehensive preliminary tests using commercial human blood verified the robustness of each step prior to large-scale production. Increasing the EDTA concentration to 3.6 mg g^{-1} prevented coagulation without altering THg or MeHg levels. Freeze-drying with stepwise temperature elevation (−40 °C → −10 °C → 20 °C) yielded a homogeneous and easily reconstituted dried matrix with reduced moisture content, ensuring biological stability. Sterilization at 21 kGy produced no measurable changes in THg or MeHg concentrations, consistent with the reported stability of

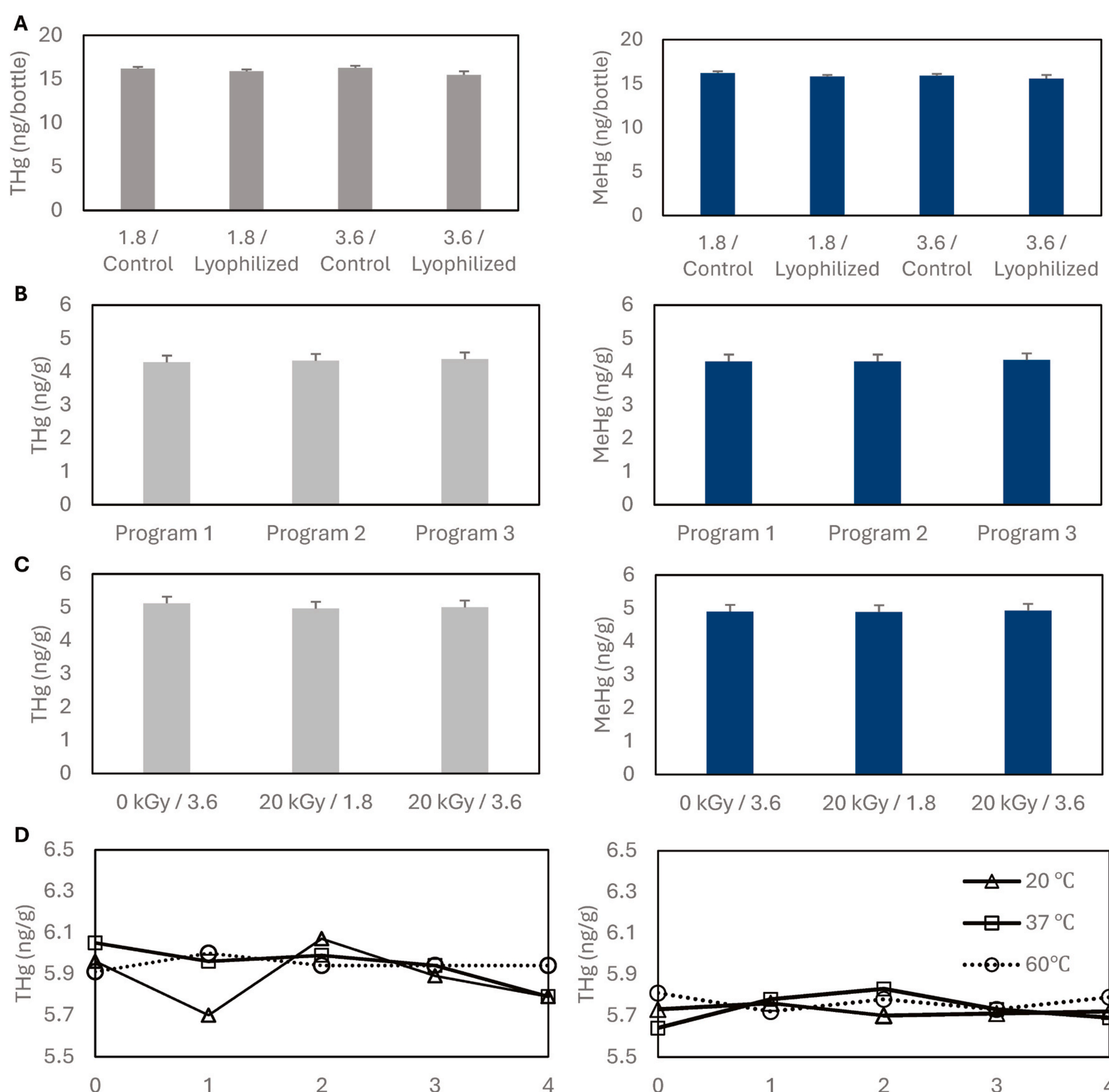

Fig. 2. Optimization of preparation and preservation conditions for the freeze-dried blood CRM. (A) Effect of EDTA-2K concentration (1.8 vs 3.6 mg g^{-1}) on THg and MeHg (as Hg) before and after lyophilization, evaluated on a mass basis (ng per bottle). (B) Comparison of three freeze-drying programs differing in the secondary drying temperature and duration (20 °C for 5 h, 10 °C for 10 h, 5 °C for 20 h). Program 1 (20 °C for 5 h) was selected as the optimized condition owing to efficient drying and stable recoveries. (C) Effect of gamma-ray sterilization (0 and 20 kGy) on THg and MeHg concentrations in freeze-dried blood containing different EDTA-2K levels. No significant difference was observed between irradiated and non-irradiated samples. (D) Short-term stability of gamma-sterilized (21 kGy) samples stored at 4, 20, and 37 °C for 0–4 weeks. Bars show mean ± SD ($n = 5$) for A–C, and $n = 1$ per time point for D. No time-dependent change was detected, confirming excellent short-term stability.

mercury species up to 50 kGy [18]. A pilot 4-week test (4 °C, 20 °C, 37 °C) confirmed excellent short-term stability (<2.5% variation). These results demonstrated the technical feasibility of producing a freeze-dried, ambient-stable blood CRM (NIMD-04), as summarized in Fig. 2.

3.2. Homogeneity

Homogeneity testing showed that bottle-to-bottle variation was generally small for all analytes. Twenty aliquots (duplicate subsamples taken from ten vials) were analyzed for THg, MeHg, and seven trace elements using the validated methods described in Section 2.7. One-way ANOVA was used to estimate the between-bottle (s_bb) and within-bottle (s_r) components. When ANOVA yielded a negative estimate of Sbb^2, the value was set to zero and replaced by the corresponding Ubb value, following ISO Guide 35:2017 [7] and the updated ISO 33405:2024 framework. For THg and MeHg, the between-bottle relative standard deviations RSD(Sbb) were below 3%, and for most trace elements they were remained below 5%. Mn exhibited largest between-bottle component, reflected in a relatively high RSD (s_bb), although the within-bottle repeatability remained sufficiently small. For the certified analytes, the between-bottle component contributed <5% to the combined uncertainty, indicating that NIMD-04 possesses adequate between-bottle uniformity for value assignment. Supplementary Table S1 summarizes the homogeneity results.

3.3. Short-term stability

Isochronous short-term tests confirmed that the material remained stable for at least 4 weeks at temperatures up to 60 °C, supporting ambient transport. Samples stored at 20, 37, and 60 °C showed no significant trends ($|b_1| < t_{0.95} \times s(b_1)$); relative uncertainties (u_sts/b_0) remained within 10% for all elements. Slight variability in Mn (≈17% at 37 °C) was attributed to random fluctuation rather than systematic degradation. These results, summarized in Table 1, indicate that the CRM can be shipped without temperature control under tropical conditions.

3.4. Long-term stability

Long-term isochronous testing indicated no measurable degradation of THg, MeHg, or trace elements during 12 months of storage at ≤ −20 °C. Regression slopes (b_1) for all analytes were statistically insignificant ($p > 0.05$). Relative long-term uncertainties (u_lts/b_0) were 1.1–9.6%, except for Mn at −20 °C (≈28%), reflecting random variation only. No statistically significant trends were observed for any analyte at either −20 °C or −80 °C during the 12-month storage period. These results support a recommended shelf life of at least one year when the CRM is stored below −20 °C.

3.5. Interlaboratory characterization

Interlaboratory characterization involving 14 institutions contributed to the assignment of certified values and demonstrated method-independent consistency across diverse analytical platforms. Each laboratory received three to five vials of NIMD-04 and a Seronorm® control solution for accuracy verification. MeHg was analyzed by nine laboratories, which applied techniques based on GC-CVAFS, HPLC-ICP-MS, GC-ECD, or TD-AAS, yielding comparable results across laboratories employing either ethylation–GC or alkaline-extraction–based methods. Method details are provided in Table 2, illustrating the wide but coherent diversity of analytical protocols.

Other trace elements (Pb, Cd, Mn, Se, As, Cu, Zn) were analyzed by all participating laboratories using ICP-QMS equipped with a collision/reaction cell. Sample preparation was grouped into four principal approaches—acid digestion, alkaline dilution, acidified stabilization, and ammonium hydroxide dilution—summarized in Table 3. Acid digestion and alkaline dilution were the predominant and most reproducible methods, providing consistent results across elements.

Submitted data were first screened using predefined analytical performance criteria to ensure the technical validity of the reported results. Potential statistical outliers among the remaining datasets were subsequently evaluated using Grubbs' test ($\alpha = 0.05$). The assigned values were calculated as the mean of the technically valid laboratory results. The evaluation of interlaboratory variability and uncertainty components followed the general principles described in ISO Guide 35:2017. Based on these procedures, the certified with expanded uncertainties ($k = 2$) were derived from the technically valid laboratory results and are summarized in Table 3. Six analytes (THg, MeHg, Pb, Se, Cu, Zn) were certified, while Cd, Mn, and As were reported as indicative values owing to higher variability or limited data. After technical screening, a small number of statistical outliers were identified using Grubbs' test ($\alpha = 0.05$), including one result each for Se and Zn. In the case of Zn, one additional result had already been excluded during the technical acceptance stage. These results were also evaluated considering their associated uncertainties and were found to lie outside the range expected from the assigned values.

3.6. Uncertainty budget summary

The combined standard uncertainty for each analyte was calculated as the quadratic sum of contributions from characterization, homogeneity, and stability studies. Characterization and between-bottle effects (60%–70%) were the major contributors to the uncertainty, whereas stability terms (u_lts, u_sts) were minor contributors (<10%). Expanded uncertainties ($k = 2$) for certified analytes ranged from 9% to 15%, which is typical for blood-based CRMs and comparable to values reported for international reference materials such as NIST SRM 955D A detailed breakdown of each uncertainty components is provided in Table 4.

4. Discussion

4.1. Scientific and practical significance of ambient-stable blood CRM

The development of a freeze-dried blood CRM that remains stable at ambient temperature directly addresses a long-standing technical and logistical gap in human biomonitoring. Conventional blood materials for mercury analysis must be transported and stored frozen to preserve the chemical form of mercury species. These requirements impose a prohibitive cost and logistical burden on laboratories in tropical or remote areas, where freezing capacity is often unavailable. The Minamata Convention's effectiveness evaluation demands globally comparable data, yet most CRMs are designed for laboratories with reliable cold-chain infrastructure.

In recent years, DBS techniques have shown that mercury speciation can be performed reliably without cold storage [5,19]. These studies demonstrated that MeHg in DBS remains stable for at least 1 year at room temperature, supporting the idea that refrigeration is not a prerequisite for sample integrity. However, DBS samples are field matrices intended for collection, not for traceability; they lack certified values and homogeneity testing. The present work complements DBS development by providing a matrix-matched, certified material that can validate or calibrate both conventional and DBS-based analyses.

In parallel with DBS, volumetric absorptive microsampling (VAMS) has emerged as another ambient-stable microsampling approach for trace elements. Recent validation work has shown that blood collected on Mitra® VAMS tips retains stable concentrations of several metals—including Hg—for multiple months at room temperature and even at 37 °C, underscoring a broader shift toward non–cold-chain analytical workflows suitable for tropical and resource-limited settings [6].

Globally, the need for CRMs has become urgent. Garzón et al. [4]

reported a persistent mismatch between the availability of reliable CRMs and the growing demand for harmonized mercury data following the Minamata Convention's entry into force. By producing an ambient-stable blood CRM, this study contributes to bridging that infrastructure gap and offers laboratories in developing regions access to traceable QA tools previously limited to cold-chain networks [5].

4.2. Evaluation of preparation and preservation parameters

The preparation strategy was designed to achieve chemical stability and biosafety simultaneously—a goal seldom realized in previous blood materials. Increasing the EDTA-2K concentration to 3.6 mg g^{-1} not only prevented coagulation during homogenization and dispensing but also maintained solubility upon reconstitution. This optimized chelation level limited metal complexation artifacts often encountered when EDTA is insufficiently controlled.

Controlled lyophilization ($-40 \rightarrow -10 \rightarrow 20$ °C) proved critical. Horvat and Byrne [18] observed up to 20% MeHg loss in freeze-dried blood owing to overheating and oxygen exposure. In contrast, the gentle stepwise temperature program used here minimized oxidative effects and preserved the natural MeHg/THg ratio, as confirmed in Section 3.1. Maintaining a low-oxygen environment prevented demethylation and ensured complete reconstitution, thus overcoming the degradation issues reported in the prior work.

Gamma irradiation at 21 kGy (7 kGy h^{-1} × 3 h) accomplished effective sterilization without altering mercury species. Previous studieshave shown that total and methyl mercury in whole blood remain stable after exposure to moderate gamma radiation doses, whereas substantial MeHg degradation (up to 70%) can occur in certain fish matrices at higher doses levels [18]. The dose level selected here balanced sterility and chemical integrity. Importantly, γ-irradiation ensured that the CRM is pathogen-free, enhancing both biosafety and long-term storage stability without compromising analytical accuracy. A previous study on a human hair reference material demonstrated that MeHg remained chemically stable after gamma irradiation and ambient-temperature transport [20], supporting the applicability of controlled irradiation of biological matrices.

Compared with NIST SRM 955D—frozen K_2EDTA-stabilized human blood stored below −60 °C—the present freeze-dried format eliminates the risk of species conversion during thawing and shipping and provides a safer alternative for laboratories without biosafety-level 2 containment.

4.3. Stability performance and usability in tropical shipment

Short-term and long-term stability studies demonstrated that NIMD-04 retained compositional integrity across the temperature range encountered in global transport. In the short-term isochronous test (20–60 °C, 4 weeks), no significant trends were detected for any analyte, indicating that the CRM can tolerate typical transit exposures. The slightly larger variability observed for Mn (~17% at 37 °C) reflected random dispersion rather than systematic degradation. These findings validate the practical feasibility of ambient transport under WHO-defined tropical shipment conditions (up to 37 °C) [21].

Long-term testing (12 months, ≤ −20 °C) confirmed stability comparable to that of established frozen CRMs, with relative uncertainties (u_lts/b_0) below 10%. The data support a conservative shelf life of at least one year when the CRM is stored below −20 °C. Even after accelerated aging at 60 °C, neither THg nor MeHg showed measurable decline, indicating robust retention of the chemical form. In this respect, the developed CRM provides a complementary option to SRM 955D, particularly for laboratories where ultra-low-temperature storage is difficult to maintain [22]. Maintaining such low-temperature conditions is feasible only through specialized cold-chain logistics, and small-parcel shipments of frozen materials are frequently subject to temperature excursions or delays [23].

From an operational perspective, the freeze-dried form reconstituted with 3 mL of ultrapure water allows consistent recovery and measurement without specialized handling. Uncertainties arising from reconstitution (pipetting precision, adsorption, incomplete dissolution) were shown to contribute less than 2% to the total uncertainty budget (Section 3.6). Consequently, the CRM provides a reliable standard for laboratories facing both thermal stress and infrastructural limitations.

Freeze-dried blood CRMs are also available from other producers, including ERM-DA634-636 (European Commission, JRC), which provide certified values for several trace elements including total mercury [3]. These materials have been evaluated using short-term isochronous stability studies and were shown to remain stable during transport at temperatures up to 60 °C. The stability results obtained for NIMD-04 are consistent with these findings and further confirm the robustness of freeze-dried blood matrices for ambient shipment. However, the ERM materials provide certified values only for total elemental concentrations and do not include mercury speciation such as methylmercury. NIMD-04 therefore complements the existing ERM materials by providing a freeze-dried blood CRM with certified values for both total mercury and methylmercury at concentration levels relevant to biomonitoring studies.

4.4. Comparative analytical performance

Comparative evaluation with established blood certified reference materials, including NIST SRM 955D (Level 1), a frozen blood CRM, and ERM-DA634, a freeze-dried blood CRM shows that NIMD-04 provides realistic mercury concentrations and analytical precision suitable for fish-consuming populations. The certified THg and MeHg values (6.16 μg L^{-1} and 5.46 μg L^{-1}, respectively) are five-to ten-fold higher than those of SRM 955D (Level 1) but remain within the range observed in populations with elevated seafood intake [24]. The MeHg/THg ratio (0.89) is consistent with physiological conditions, indicating that lyophilization and sterilization did not substantially alter species distribution. A comparative summary of certified and indicative values of NIMD-04 together with selected blood certified reference materials and a commercial quality-control material is shown in Table 5.

The expanded uncertainties ($k = 2$) of 10.1% for THg and 9.2% for MeHg are slightly larger than those reported for some established blood CRMs (e.g., SRM 955D, ~6%), but remain within the typical range for biological CRMs and are suitable for interlaboratory comparison and routine biomonitoring applications. Elements such as Pb, Se, Cu, and Zn showed close agreement with the selected comparison materials, while Cd, Mn, and As were assigned as indicative values because their measurements in NIMD-04 showed higher interlaboratory variability. This transparent, tiered value-assignment approach allows users to select analytes according to their analytical scope and intended use. The generally consistent results obtained across fourteen laboratories, multiple analytical techniques, and varied digestion systems (Tables 2 and 3) indicate that the assigned values are applicable across a range of commonly used analytical platforms.

A dedicated commutability study using individual clinical blood samples and multiple analytical methods was not performed in this study. The analytical techniques applied in this study (e.g., TDA-AAS, ICP-MS, and chromatographic methods for MeHg) are widely used in routine biomonitoring laboratories. The material was prepared from pooled human whole blood without artificial fortification, and freeze-drying was applied only to improve stability and transportability. After reconstitution, the material therefore retains a blood-derived matrix.

Although this does not constitute a formal demonstration of commutability, the generally consistent results obtained across commonly used analytical techniques suggest that the material may behave similarly to routine blood samples under typical analytical conditions. In particular, consistent results were obtained across widely used analytical approaches for both total elements and methylmercury, including

Table 5
Comparison of certified or indicative values of NIMD-04 with selected blood certified reference materials and a commercial quality-control material. For Seronorm L-1, the values shown are manufacturer-provided target values and associated uncertainty or acceptable range; this material is a commercial quality-control material, not a certified reference material.

			NIMD-04			NIST SRM 955D (Level 1)			ERM-DA634			Seronorm L-1 (QC material)		
Element	Unit	Type	Value	Uexp	Urel (%)	Value	Uexp	Urel (%)	Value	Uexp	Urel (%)	Value	Uexp	Urel (%)
THg	μg L^{-1}	Certified	6.16	0.62	10.1	1.373	0.081	5.9	1.6	0.3	18.8	1.63	0.33	20.2
MeHg as Hg	μg L^{-1}	Certified	5.46	0.50	9.2	0.626	0.020	3.2	-	-	-	1.24	0.50	-
Pb	μg L^{-1}	Certified	6.76	0.60	8.9	14.800	0.26	1.8	18.6	1.7	9.1	10.3	2.1	20.4
Se	mg L^{-1}	Certified	0.182	0.028	15.4	0.2066	0.0095	4.6	-	-	-	0.067	0.020	29.9
Cu	mg L^{-1}	Certified	0.667	0.090	13.5	-	-	13.3	-	-	-	0.65	0.13	20
Zn	mg L^{-1}	Certified	4.82	0.55	11.4	-	-	5.9	-	-	-	4.5	0.9	20
Cd	μg L^{-1}	Indicative	0.88	0.13	14.8	0.326	0.010	3.1	1.29	0.09	7.0	0.29	0.06	20.7
Mn	μg L^{-1}	Indicative	18	8.5	47.2	48.8	1.3	2.7	-	-	-	16.3	3.3	20.2
As	μg L^{-1}	Indicative	3.4	0.58	17.1	5.31	0.76	14.3	-	-	-	2.5	0.5	20

acid digestion and alkaline solubilization methods (e.g., TMAH-based procedures; Table 3), as well as instrumental techniques such as CV-AAS, ICP-MS, and chromatographic methods (Table 2). For example, dilution-based ICP-MS methods, when appropriately optimized, have been reported to yield results broadly consistent with acid digestion methods, suggesting that method-dependent differences may be minimized under suitable conditions. In contrast, one laboratory using ammonium hydroxide (NH_4OH)-based dilution without acid digestion (Table 3) reported inconsistent results for several elements, indicating that the observed behavior may depend on the applied sample-preparation procedure. Therefore, commutability is considered to be reasonably assumed based on methodological consistency, but has not been formally demonstrated, and method-dependent differences may occur depending on the analytical approach.

4.5. *Uncertainty evaluation and metrological conformity*

The uncertainty budget demonstrates that NIMD-04 satisfies ISO 17034 [8] and ISO Guide 35 [7] requirements for CRMs. The combined uncertainty u_c was derived from characterization (u_char), between-bottle (u_bb), and stability components (u_sts, u_lts), with 60%–70% of the total uncertainty arising from the first two factors and <10% from stability terms. This distribution indicates that the lyophilization and storage procedures introduced negligible additional uncertainty.

Expanded uncertainties ($k = 2$) of 9%–15% for certified analytes fall within the range of leading biological CRMs such as NIST SRM 955D and IAEA 085 (typically 5%–8%). These values are sufficiently low for use in interlaboratory comparisons and method validation under the Minamata Convention's QA/QC framework. The conservative assignment of certain elements as reference rather than certified values ensures transparent traceability and aligns with best practices in metrological certification.

4.6. *Broader implications and future perspectives*

The present work builds upon previous efforts to develop matrix-matched mercury reference materials, including a human hair reference material exhibiting MeHg stability after gamma irradiation and ambient-temperature transport [20] and a subsequently certified human hair CRM for total mercury, methylmercury, and trace elements [25]. Together, these materials constitute a complementary suite of biological CRMs—hair for external exposure assessment and blood for internal dose evaluation—supporting harmonized biomonitoring under the Minamata Convention framework.

The creation of an ambient-stable blood CRM extends beyond technical innovation; it redefines an implicit consensus that blood CRMs must be cold-chain-dependent. By providing a reliable, logistically feasible alternative, NIMD-04 enhances analytical equity among laboratories worldwide. This material directly supports Article 19 of the Minamata Convention, which calls for strengthening scientific and technical capacity for mercury monitoring, particularly in developing regions.

More than a decade ago, Ibáñez-Palomino et al. [26] reviewed CRMs for mercury speciation and concluded that they "do not fully meet user needs," particularly for analyzing biological fluids such as blood and urine, and for laboratories lacking low-temperature storage facilities. The present study directly responds to this long-standing limitation by providing a freeze-dried, sterile, and chemically stable blood CRM that can be stored and shipped at ambient temperature. This therefore addresses a need identified over 10 years ago into a practical, metrologically traceable solution.

From a QA standpoint, the CRM fills a recognized gap in global PT schemes in which blood matrices are underrepresented. It will enable routine control-charting of biological mercury analyses and facilitate integration of HBM data across national networks. Looking forward, the same design principles—controlled lyophilization, moderate irradiation, and fixed-volume reconstitution— may inform the development of analogous ambient-stable CRMs for other biological fluids, such as serum and urine.

Future work should include evaluation using donor pools with diverse exposure backgrounds, assessment of higher mercury levels relevant to highly exposed groups, and extended ambient-stability studies over multi-year timescales. International registration in COMAR and coordinated distribution via UNEP/WHO partnerships would promote adoption and capacity building. Collectively, these efforts advance analytical chemistry toward a globally inclusive infrastructure for mercury biomonitoring.

5. Conclusions

This study established a freeze-dried, ambient-stable blood CRM (NIMD-04) with verified homogeneity, stability, and traceable certified values for THg and MeHg. Short-term transport under ambient conditions and storage at ≤ -20 °C for at least 1 year caused no measurable degradation. Interlaboratory characterization involving 14 institutions yielded certified values of 9%–15% for THg, MeHg, Pb, Se, Cu, and Zn with expanded uncertainties ($k = 2$) and indicative values for Cd, Mn, and As. The material provides concentration ranges of THg and MeHg representative of fish-consuming populations and is fully compatible with existing analytical techniques. By eliminating reliance on cold-chain logistics, NIMD-04 redefines the paradigm of blood CRMs and provides a practical, equitable foundation for global mercury biomonitoring under the Minamata Convention. This work also fulfills a need first articulated more than a decade ago [26] by delivering a practical, ambient-stable blood CRM that overcomes the cold-chain dependence of earlier materials.

CRediT authorship contribution statement

Koichi Haraguchi: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mineshi Sakamoto:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Hiromitsu Nagasaka:** Formal analysis, Investigation, Writing – review & editing. **Keisuke Uchida:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Yasunori Ito:** Investigation, Validation, Writing – review & editing. **Milena Horvat:** Methodology, Supervision, Writing – review & editing. **Matthew D. Rand:** Formal analysis, Methodology, Writing – review & editing. **Ciprian Mihai Cirtiu:** Formal analysis, Methodology, Writing – review & editing. **Emmanuel Yumvihoze:** Formal analysis, Methodology, Writing – review & editing. **Hitoshi Kodamatani:** Formal analysis, Methodology, Writing – review & editing. **Megumi Yamamoto:** Formal analysis, Writing – review & editing. **Miyuki Iwai-Shimada:** Formal analysis, Methodology, Writing – review & editing. **Hung T. Duong:** Formal analysis, Writing – review & editing. **Naoko Hishida:** Formal analysis, Writing – review & editing. **Maxim Slyadnev:** Formal analysis, Writing – review & editing. **Ermira Begu:** Formal analysis, Writing – review & editing. **Polona Klemenčič:** Formal analysis, Writing – review & editing. **Adna Alilović:** Formal analysis, Writing – review & editing. **Kenta Iwai:** Formal analysis, Writing – review & editing. **Van Thi Nguyen:** Formal analysis, Writing – review & editing. **Masaaki Nakamura:** Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Glossary

Analytical terms

CRM	Certified Reference Material
THg	Total Mercury
MeHg	Methylmercury
EDTA-2K	Dipotassium ethylenediaminetetraacetate (anticoagulant)
TDA-AAS	Thermal Decomposition–Amalgamation Atomic Absorption Spectrometry
CV-AAS	Cold Vapor Atomic Absorption Spectrometry
CV-AFS	Cold Vapor Atomic Fluorescence Spectrometry
ICP-QMS	Inductively Coupled Plasma Quadrupole Mass Spectrometry
GC-ECD	Gas Chromatography–Electron Capture Detection
HPLC-ICP-MS	High-Performance Liquid Chromatography coupled with ICP-MS

Stability & homogeneity terms

s_ts	Short-Term Stability
l_ts	Long-Term Stability
s_r	Within-bottle standard deviation
s_bb	Between-bottle standard deviation
S_bcr	Combined between- and within-bottle variability

Uncertainty & statistics

ANOVA	Analysis of Variance
u_char	Uncertainty from characterization
u_bb	Uncertainty from between-bottle effects
u_sts	Uncertainty from short-term stability
u_lts	Uncertainty from long-term stability
u_c	Combined standard uncertainty
U (k = 2)	Expanded uncertainty *(k = 2)*

Regulatory / monitoring

HBM	Human Biomonitoring
ILC	Interlaboratory Comparison
PT	Proficiency Testing

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2026.345580.

Data availability

Data will be made available on request.

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