National Institute for Minamata Disease
Ministry of the Environment, Japan

# Reference Material Certificate

## NIMD-04

### Human Whole Blood

This reference material is freeze-dried human blood (whole blood) produced in accordance with ISO Guide 35 (JIS Q 0035) . NIMD-04 can be used to validate analytical methods or equipment, or to operate quality management in quantitative analyses of trace elements in blood samples.

1. Certified and indicative values

Certified values of NIMD-04 shown below are traceable to the International System of Units (SI). Expanded uncertainties of certified values are obtained by multiplying standard uncertainties by a coverage factor $k = 2$.

| Class | Elements | Unit | Value | Expanded uncertainty | Analytical method (refer to annotations below) |
|---|---|---|---|---|---|
| Certified value | Methyl mercury (Me-Hg) | μg/L | 5.46 | 0.50 | 1),2),3),4),5) |
| | Total mercury (T-Hg) | μg/L | 6.16 | 0.62 | 6),7),8),9) |
| | Lead (Pb) | μg/L | 6.76 | 0.60 | 9) |
| | Selenium (Se) | mg/L | 0.182 | 0.028 | 9) |
| | Copper (Cu) | mg/L | 0.667 | 0.090 | 9) |
| | Zinc (Zn) | mg/L | 4.82 | 0.55 | 9) |
| Indicative value | Cadmium (Cd) | μg/L | 0.88 | 0.13 | - |
| | Manganese (Mn) | μg/L | 18 | 8.5 | - |
| | Arsenic (As) | μg/L | 3.4 | 0.58 | - |

Analytical methods:
1) Gas Chromatography-Electron Capture Detector (GC-ECD)
2) High Performance Liquid Chromatography-Chemiluminescence (HPLC-Chemiluminescence)
3) Ethylation-Gas Chromatography-Cold Vapor Atomic Fluorescence (Ethylation-GC-CVAF)
4) High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS)
5) Solvent Extraction-Thermal Decomposition Atomic Absorption (Solvent extraction-TDAA)
6) Thermal Decomposition Atomic Absorption (TDAA)
7) Cold Vapor Atomic Absorption (CVAA)
8) Cold Vapor Atomic Fluorescence (CVAF)
9) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

2. Instructions for use

(1) Tap the sample bottle lightly to confirm that the contents are at the bottom of the sample bottle.

(2) Remove the screw cap and rubber stopper.

(3) Add 3.0 mL of ultrapure water and gently rotate or shake the bottle for at least 30 minutes to prepare the reconstituted blood. (Time should be adjusted so that the contents are completely dissolved. Also, avoid foaming.)

3. Characterization and value assignment

An inter-laboratory study based on instructions and a reporting format satisfying the JIS Q 0035:2008 criteria was conducted for characterization by 14 laboratories*. Value assignment was performed based on documented test procedures and statistical handlings on outliers and approach to assign uncertainty.

*: Includes the number of test results conducted at different laboratories within the same institute.

4. Period of validity

The period of validity for this reference material is one year from the date of shipment, provided that the conditions described in "7. Instruction on storage" are met. Storage at -80 degrees Celsius is expected to enable longer-term preservation; in such cases, the corresponding expiration date will be posted on the producer's website （http://nimd.env.go.jp/english/）.

5. Product form

This reference material is a freeze-dried sample of 3 mL whole blood, sterilized by γ-ray irradiation and sealed in a brown glass bottle.

6. Homogeneity

After subdivided into 800 bottles, 20 were selected by stratified sampling, 10 of which were used to analyzed for total mercury, methylmercury, and the remaining 10 bottles were used for lead, cadmium, manganese, selenium, arsenic, copper, and zinc. Homogeneity of sample was evaluated by analysis of variance. The value of uncertainty derived from homogeneity was within the uncertainty of certified value. Thus, the homogeneity of NIMD-04 is ensured within the range of uncertainty of certified value.

7. Instruction on storage

Store unopened NIMD-04 in a clean, light-shielded place below -20 degrees Celsius.

8. Instruction for handling and usage

(1) Although NIMD-04 is sterilized with γ-ray irradiation, consider the potential risk as a source of diseases.
(2) To homogenize the content, shake the bottle well before use.
(3) Before aliquoting samples for analysis, they should be prepared according to "2. Instructions for use".

9. Production method

To produce NIMD-04, blood materials were collected from general population in Japan, mixed and homogenized, and packed into glass bottles before freeze-dried. The bottles were filled with nitrogen gas to prevent deterioration of the samples. All processing operations were conducted by IDEA Consulting, Inc.

10. Reference material producer

NIMD-04 was produced by National Institute for Minamata Disease, Ministry of the Environment, Japan.

11. Participants

The values of NIMD-04 were characterized through an inter-laboratory study conducted by institutes listed below.

| No. | Institution | Country |
|---|---|---|
| 1 | IDEA Consultants, Inc. | Japan |
| 2 | Institut national de santé publique du Québec | Canada |
| 3 | Jožef Stefan Institute | Slovenia |
| 4 | Kagoshima Uniersity | Japan |
| 5 | Lumex Instruments | Canada |
| 6 | National Institute for Environmental Studies (NIES) | Japan |
| 7 | National Institute Minamata Disease (NIMD) | Japan |
| 8 | Nippon Instruments Corporation (NIC) | Japan |
| 9 | SHIMADZU Techno-Research, Inc. | Japan |
| 10 | University of Ottawa | Canada |
| 11 | University of Rochester School of Medicine and Dentistry | US |
| 12 | Vietnam Academy of Science and Technology | Vietnam |

Institutes are in alphabetical order

12. Access to information

Information on any important revision concerning NIMD-04 will be announced at the web site of the producer (http://nimd.env.go.jp/english/). Technical information on NIMD-04 can be acquired from the contact address below.

13. Replicate of certificate

Indicate as copy when replicating this certificate.

April 10, 2025
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Certification Revision History
April 10, 2025 (Updated expiration conditions: storage temperature and unopened status)
November 29, 2024 (Change of expiration)
December 1, 2023 (Original certificate date)