Supplementary material

Organization of the proficiency tests

In order to provide a harmonised approach for the organization and evaluation of the different ICI/EQUAS exercises in HBM4EU, protocols were drafted and described in standard operating procedures (SOPs). These SOPs were based on existing protocols originating from ISO17043-accredited organisations, and included detailed instructions for all aspects of the QA/QC programme, such as the description of the roles and responsibilities of the organisers, timeline of the exercises, definitions of different terms or templates for communication with the participants and reporting of the results. Additional SOPs were drafted for the preparation and characterization of control materials and for the evaluation of participants' results. Details of the preparation of the various control materials are provided elsewhere. The characterization of the control materials included homogeneity and stability testing.

References

Esteban López, M., Göen, T., Mol, H., Nübler, S., Haji-Abbas-Zarrabi, K., Koch, H., Dvorakova, D., Hajslova, J., Antignac, J-P., Vaccher, V., Elbers, I, Thomsen, C., Vorkamp, K., Pedraza–Díaz, S., Kolossa-Gehring, Castaño, A., 2021. The European Human Biomonitorng platform - design and implemention of a QA/QC programme for selected priority chemicals. Int J Hyg Environ Health. revision submitted

Analytical procedure for the testing of control materials

Cd (U) and Cd (B) were quantified according to a published method (Schramel et al. 1999). ICP-MS (Perkin Elmer NexION 350d) was used with argon (99.999 vol%) as the operating gas for plasma generation. An internal standard (Rhodium ICP standard in 20% HCl) was added to each eluent to a concentration of 20 µg Rh/L. For standardization, the counts of the ^{114}Cd or ^{98}Mo signal were divided by the counts of the ^{103}Rh signal. This quotient was used as a correction factor for the Cd signal. Samples were diluted with acid solution (urine) or basic solution (blood) before autosampler injection. The specific dilution factor as a ratio of the final volume to the initial volume was 1:10 for Cd (U) and 1:20 for Cd (B). For urine calibration, the respective stock solution (ClinCal urine calibrator, 19.1 µg Cd/l, Recipe) was diluted in urine (matrix-based) to five different

concentrations. (1:200, 1:100, 1:20, 1:10 and 1:5). For blood calibration, the respective stock solution (multi-element standard XXI, 10 mg/l, Merck) was diluted in blood (matrix-based) to six different concentrations (1:20000, 1:10000, 1:2000, 1:400, 1:200 and 1:100). LOD and LOQ for Cd (U) and Cd (B) were calculated from the standard deviation of the calibration function obtained according to DIN 32645 (Bader et al., 2010). For urine, an equidistant 5-point calibration (0.096-3.840 µg Cd/l) was prepared and processed together with a blank value (pool urine without doping). For blood, an equidistant 6-point calibration (0.500-100 µg Cd/l) was prepared and processed together with a blank value (pooled blood without spiking). The molybdenum (Mo) background levels were also detected in each CM using the same method.

References

Bader, M., Barr, D, Göen, T., Schaller, K.H., Scherer, G., Angerer, J. (2010) Reliabiality criterial for analytical methods. The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods, Vol. 12, Deutsche Forschungsgemeinschaft (DFG), Wiley-VCH, Weinheim, pp 55-101; https://onlinelibrary.wiley.com/doi/pdf/10.1002/3527600418.bireliabe0012

Schramel, P., Wendler, I., Dunemann, L., Fleischmann, M., Emmons, H. (1999) Antimony, lead, cadmium, platinum, mercury, tellurium, thallium, bismuth, tungsten, tin. Determination in urine. In: Angerer, J., Schaller, K., Greims, H. (Eds.), Analysis of Hazardous Substances in Biological Materials, Vol. 6. Wiley-VCH, Weinheim, pp. 79–109

Supplementary Tables

Suppl. Table 1 Spiking concentrations of the CM for Cd (U) and Cd (B) in the different ICI/EQUAS rounds

Target concentration [µg/l]	Cd $(U)_{low}$	Cd $(U)_{high}$	Cd $(B)_{low}$	Cd $(B)_{high}$
Round 1*	0.100	0.350	0.200	0.500
Round 2	0.000	0.180	0.120	0.720
Round 3	0.040	0.140	0.180	0.320
Round 4	0.020	0.100	0.120	0.370

*the native content of Cd was 0.11 µg/l in the urine CM and 0.01 µg/l in the blood CM

Suppl. Table 2 Results of the stability testing for Cd (U) and Cd (B)

Mean stability concentration ± SD [µg/l]	Cd $(U)_{low}$		Cd $(U)_{high}$		Cd $(B)_{low}$		Cd $(B)_{high}$	
	-80°C	**-18°C**	**-80°C**	**-18°C**	**-80°C**	**-18°C**	**-80°C**	**-18°C**
Round 1*	0.195* ± 0.014	0.230* ± 0.008	0.452* ± 0.019	0.472* ± 0.010	0.236* ± 0.023	0.213* ± 0.021	0.505* ± 0.025	0.493* ± 0.018
Round 2	0.051 ± 0.001	0.051 ± 0.001	0.233 ± 0.015	0.228 ± 0.012	0.080 ± 0.005	0.080 ± 0.010	0.760 ± 0.023	0.760 ± 0.021
Round 3	0.092 ± 0.002	0.093 ± 0.004	0.196 ± 0.005	0.194 ± 0.009	0.201 ± 0.022	0.189 ± 0.010	0.337 ± 0.025	0.327 ± 0.011
Round 4	0.070 ± 0.009	0.072 ± 0.008	0.147 ± 0.014	0.143 ± 0.018	0.150 ± 0.009	0.153 ± 0.019	0.387 ± 0.015	0.385 ± 0.014

* in round 1, stability was assessed slightly differently: ten samples were analysed on the day of sample preparation (see values in column -80°C) and then compared to the analysis of ten samples that had been stored at -18°C for four weeks.

Suppl. Table 3 Method details of participants and experts analysing Cd (U)

	applied instrument		use of internal standard (IS)			Mo INT compensated	
Cd (U) participants	**ICP-MS**	**AAS**	**yes, response normalised to IS**	**yes, response not normalised to IS**	**no**	**yes**	**no**
Round 1	90%	10%	71%	10%	19%	38%	62%
Round 2	94%	6%	81%	3%	17%	39%	61%
Round 3	95%	5%	80%	5%	15%	39%	61%
Round 4	95%	5%	89%	5%	5%	50%	50%
Cd (U) experts	**ICP-MS**	**AAS**	**yes, response normalised to IS**	**yes, response not normalised to IS**	**no**	**yes**	**no**
Round 2	100%	0%	100%	0%	0%	60%	40%
Round 3	100%	0%	100%	0%	0%	60%	40%
Round 4	100%	0%	100%	0%	0%	50%	50%

Suppl. Table 4 Method details of participants and experts analysing Cd (B)

	applied instrument		use of internal standard (IS)			Mo INT compensated	
Cd (B) participants	**ICP-MS**	**AAS**	**yes, response normalised to IS**	**yes, response not normalised to IS**	**no**	**yes**	**no**
Round 1	95%	5%	78%	11%	11%	27%	73%
Round 2	90%	10%	80%	7%	13%	44%	56%
Round 3	94%	6%	79%	6%	15%	38%	62%
Round 4	89%	11%	94%	0%	6%	50%	50%
Cd (B) experts	**ICP-MS**	**AAS**	**yes, response normalised to IS**	**yes, response not normalised to IS**	**no**	**yes**	**no**
Round 2	100%	0%	100%	0%	0%	40%	60%
Round 3	100%	0%	100%	0%	0%	40%	60%
Round 4	100%	0%	100%	0%	0%	50%	50%

Suppl. Table 5 Interpretation of results reported as `<LOQ`

Calculated Z-score from LOQ (LOQ-Z)	Interpretation
LOQ-Z ≤ -3	Result was considered as clear false negative result, as the laboratory should have been able to detect and quantify the biomarker with the indicated LOQ
-3 < LOQ-Z < -2	Result was considered as possible false negative result, as the laboratory should have been able to detect and quantify the biomarker with the indicated LOQ
-2 ≤ LOQ-Z ≤ 2	-2 to 0: LOQ < assigned value, but result cannot be classified as false negative. 0 to 2: LOQ > assigned value, but not still considered within acceptable range relative to the assigned value.
2 < LOQ-Z < 3	LOQ was relatively high compared to the other laboratories
LOQ-Z ≥ 3	LOQ was considered too high compared to the other laboratories

Suppl. Table 6 Overview of the <LOQ results for Cd (U) and Cd (B)

				LOQ << assigned value (false negative)	LOQ < assigned value	LOQ within acceptable range to the assigned value	LOQ > assigned value	LOQ >> consensus/assigned value
Cd (U)	**round**	**CM**	**Number of <LOQ results**	**LOQ-Z ≤ -3**	**-3 < LOQ-Z < -2**	**-2 ≤ LOQ-Z ≤ 2**	**2 < LOQ-Z < 3**	**LOQ-Z ≥ 3**
	1 (ICI)	**low**	1					1
		high	1					1
	2 (EQUAS)	**low**	5			4		1
		high						
	3 (EQUAS)	**low**	2			2		
		high	1		1			
	4 (EQUAS)	**low**	1			1		
		high						
	Total number		**11**	**0**	**1**	**6**	**0**	**4**
Cd (B)	**round**	**CM**	**Number of <LOQ results**	**LOQ-Z ≤ -3**	**-3 < LOQ-Z < -2**	**-2 ≤ LOQ-Z ≤ 2**	**2 < LOQ-Z < 3**	**LOQ-Z ≥ 3**
	1 (ICI)	**low**	1			1		
		high	1			1		
	2 (EQUAS)	**low**	8			3		5
		high	1			1		
	3 (EQUAS)	**low**	5	1		3		1
		high	2			1		1
	4 (EQUAS)	**low**	2	1		1		
		high	1			1		
	Total number		**21**	**2**	**0**	**12**	**0**	**7**

Suppl. Table 7 Study RSD_R of all HBM4EU approved laboratories for Cd (U) and Cd (B)

studyRSD$_R$	**Cd (U)$_{low}$**	**Cd (U)$_{high}$**	**Cd (B)$_{low}$**	**Cd (B)$_{high}$**
Round 1	14%	12%	10%	10%
Round 2	35%	12%	21%	7%
Round 3	10%	12%	16%	11%
Round 4	17%	8%	14%	8%
Mean all rounds	**19%**	**11%**	**15%**	**9%**

Suppl. Table 8 Ion mass monitoring and MoO suppression measures in ICP-MS laboratories (Percentage[#])

	Round	number of ICP-MS laboratories	Masses monitored				Reagent gas				
			111	114	111 and 114	n.s.	He	H_2 and He	nogas	methane	n.s.
Cd (B) participants	**1**	**14**	50%	19%	25%	6%	25%	6%	50%	6%	13%
	2	**26**	67%	18%	11%	4%	33%	4%	48%	4%	11%
	3	**27**	61%	16%	10%	13%	45%	3%	30%	3%	19%
	4	**13**	65%	0%	21%	14%	50%	0%	43%	0%	7%
Cd (B) experts	**2**	**6**	0%	40%	20%	40%	20%	0%	40%	0%	40%
	3	**6**	33%	33%	17%	17%	33%	17%	17%	0%	33%
	4	**5**	25%	25%	50%	0%	50%	25%	25%	0%	0%
Cd (U) participants	**1**	**17**	43%	26%	26%	5%	26%	0%	43%	5%	26%
	2	**28**	50%	25%	19%	6%	28%	0%	45%	7%	20%
	3	**37**	59%	11%	8%	22%	35%	3%	37%	3%	22%
	4	**18**	65%	0%	24%	11%	41%	6%	53%	0%	0%
Cd (U) experts	**2**	**6**	15%	22%	40%	23%	30%	0%	50%	0%	20%
	3	**6**	17%	50%	16%	17%	29%	13%	29%	0%	29%
	4	**5**	25%	25%	50%	0%	50%	25%	25%	0%	0%

[#] Percentages of ICP-MS laboratories which indicated specific ion mass monitoring and molybdenum oxide suppression measures

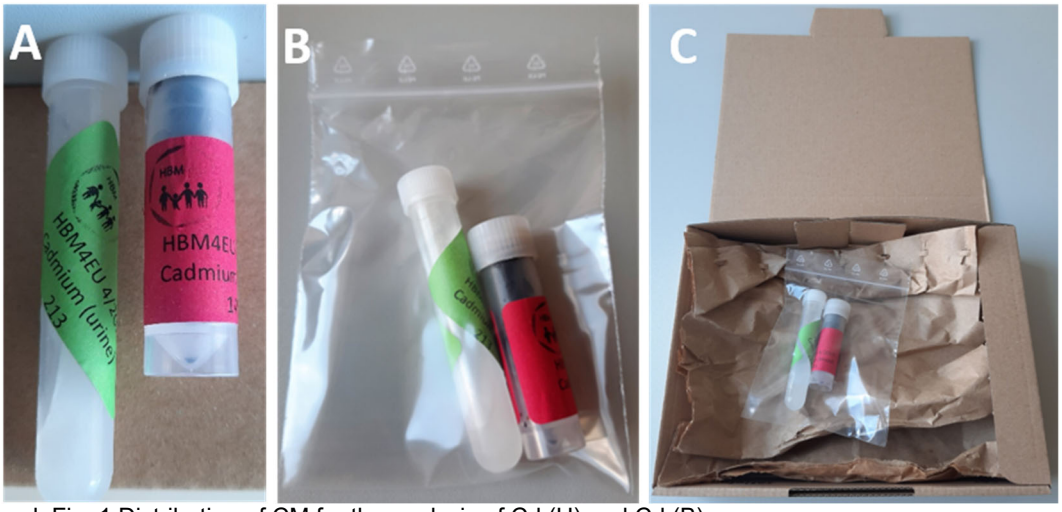
Suppl. Table 9 List of participating laboratories

Country	Laboratory group	Institution
Austria	Testing Laboratory for environmental analysis, GMO and fuel analysis	Environment Agency Austria
Belgium	Laboratory of Toxicology	CHU Liège
Belgium	WD Chemical and Physical Health Risks Unit Trace Elements	Sciensano
Belgium	Laboratory of Industrial and Environmental Toxicology	Cliniques Universitaires Saint-Luc, Université catholique de Louvain
Belgium	Analytical, Environmental and Geochemistry (AMGC)	Vrije Universiteit Brussel (VUB)
Cyprus	Water and Health Laboratory	Cyprus International Institute for Environmental and Public Health, Cyprus University of Technology
Czech Republic	Trace Analytical Laboratory	Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University
Denmark	Department of Bioscience	Aarhus University
Finland	Biomonitoring Laboratory of FIOH	Finnish Institute of Occupational Health (FIOH)
France	LERES	French School of Public Health - EHESP, Ecole des Hautes Etudes en Santé Publique
France	Department Toxicology and Biomonitoring	INRS
France	TOXILABO	TOXILABO
France	Pharmacology and Toxicology laboratory	University Hospital Limoges
Germany	Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA)	Ruhr University Bochum
Germany	Forensische und Klinische Toxikologie	Labor Dr. Wisplinghoff
Germany	Institute and Clinic for Occupational, Social and Environmental Medicine	University Hospital LMU Munich
Germany	Fraunhofer Institute for Molecular Biology and Applied Ecology	Fraunhofer Institute for Molecular Biology and Applied Ecology
Germany*	Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM)	Friedrich-Alexander University Erlangen-Nürnberg
Germany	Laboratory for Toxiclogy and Immunology	Institute for Occupational and Maritime Medicine (ZfAM) University Medical Centre Hamburg-Eppendorf (UKE)
Greece	Health and Exposome Research Centre (HERACLES), Center for Transdisciplinary Research and Innovation (KEDEK)	Aristotle University of Thesaloniki
Hungary	Central Laboratory	National Public Health Center (NPHI)
Ireland	Dublin Public Analyst`s Laboratory	HSE
Italy	Dept Environment and Health	Instituto Superiore di Sanità
Italy	Laboratory of Environmental and Industrial Toxicology	University of Milan
Japan*		IDEA Consultants, Inc.
Latvia	Laboratory of Hygiene an Occupational Diseases	RSU Institute of Occupational and Environmental Health
Latvia	Laboratory of Analytical Chemistry	University of Latvia Faculty of Chemistry
Lithuania	Laboratory of Toxicology, Neuroscience Institute of Lithuanian University of Health Sciences	LSMU Lietuvos sveikatos mokslu universitetas (Lithuanian University of Health Sciences)
Luxembourg	Hygiène du Milieu et Surveillance Biologique	Laboratoire national de santé LNS
Norway	NILU-MILK	NILU-Norwegian Institute for Air Research

Poland	Metal Analysis Laboratory	Nofer Institute of Occupational Medicine
Portugal	Food and Nutrition Department	Instituto Nacional de Saúde Dr. Ricardo Jorge, INSA. National Institute of Health
Portugal	Geobiotec-Geochemistry Laboratory	Department of Geosciences, University of Aveiro
Slovak Republic	Department of metallomics	Slovak Medical University
Slovak Republic	Department of Hygienic Laboratory Center	Public Health Authority of the Slovak Republic
Slovenia*	Department of Environmental Sciences	Jozef Stefan Institute
Slovenia	Center for Chemical Analysis of Food, Water and other Environmental Samples	National Laboratory of Health, Environment and Food
Spain*	Toxicologica Ambiental CNSA-ISCIII	Instituto de Salud Carlos III (ISCIII) - CNSA
Spain		Laboratorio de Salud Publica de Alicante (LSPA)
Spain	Department of Legal Medicine and Toxicology Service	School of Medicine, University of Granada
Spain	Laboratory of Toxicology	University of Las Palmas de Gran Canaria, ULPGC
Spain	Instituto de Toxicología del a Defensa (ITOXDEF)	Defense Ministry
Sweden*	Occupational and enviromental medicine	Laboratory medicine
Sweden	Metals and Health, Institute of Environmental Medicine (IMM)	Karolinska Institutet
Switzerland	Lausanne Forensic Toxicology and Chemistry Unit	University Center of Legal Medicine
UK	Biological Monitoring team	Health & Safety Laboratory
UK	Inorganic Geochemistry	British Geological Survey
USA*	Division of Environmental Health Sciences	New York State Department of Health

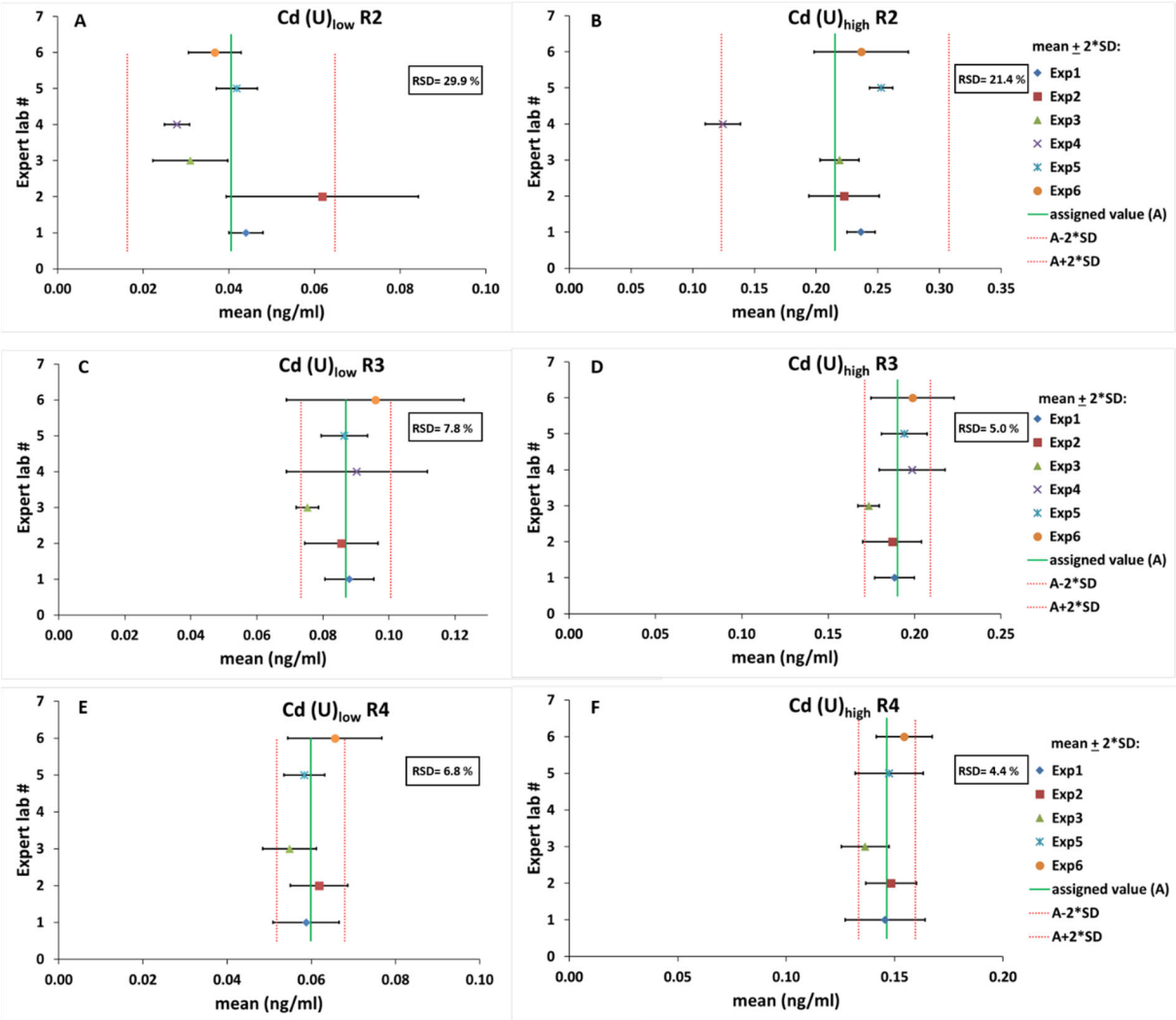
*expert laboratories

Supplementary Figures

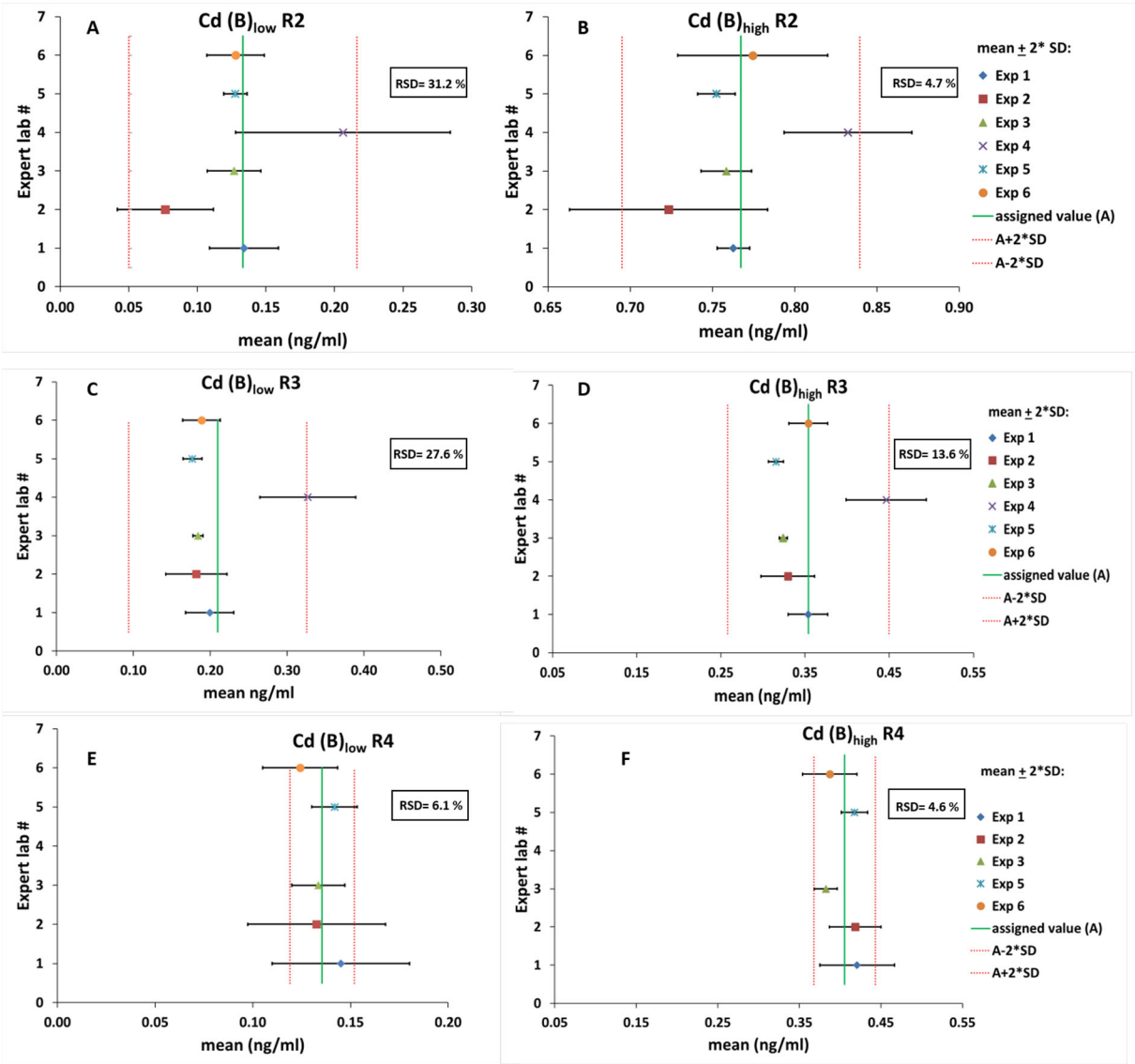


Suppl. Fig. 1 Distribution of CM for the analysis of Cd (U) and Cd (B)

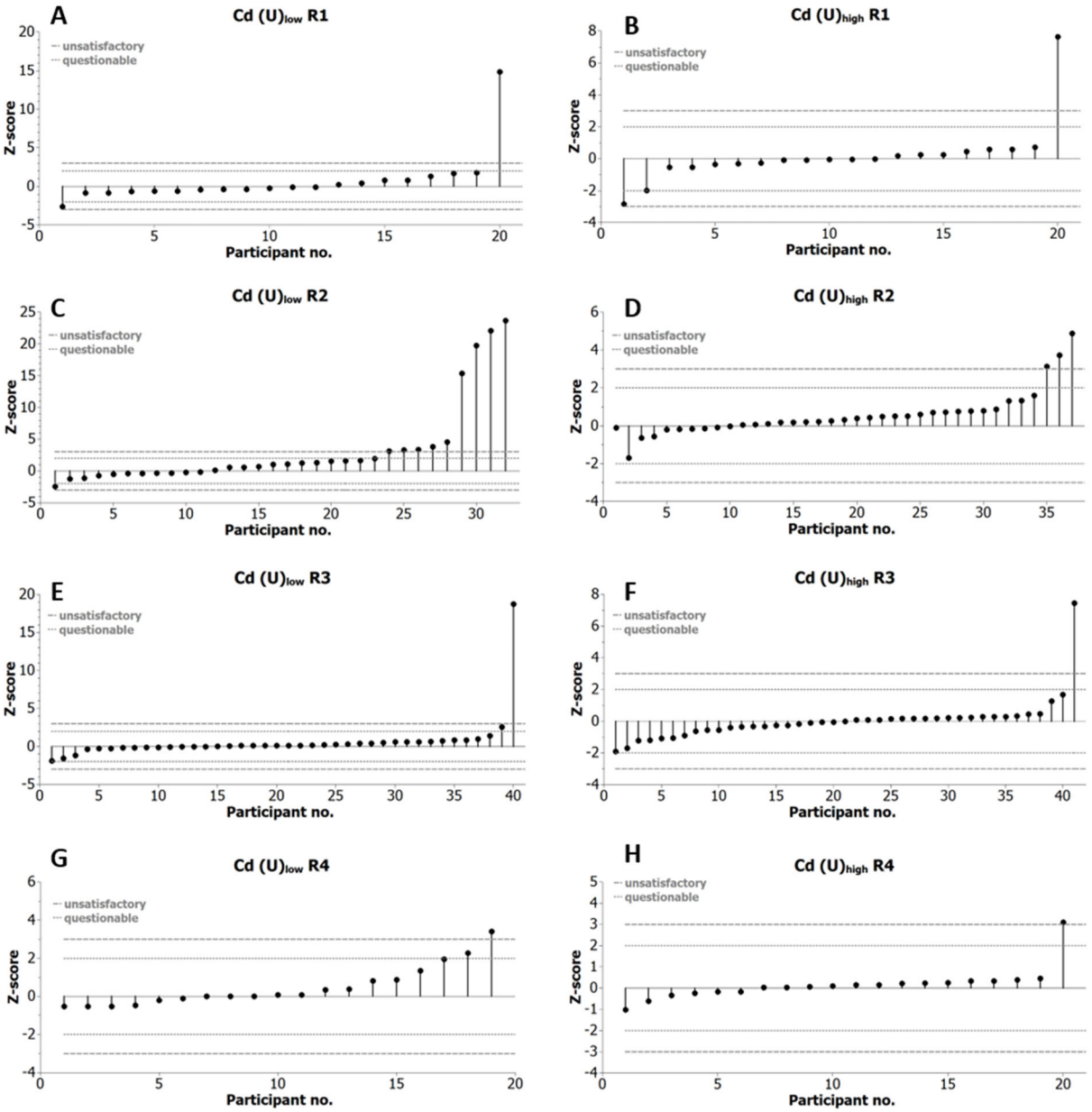
The tube with the green label contained the urinary CM and the tube with the red label the blood CM (**A**). CMs were packed and shipped to participants under ambient conditions (**B, C**).



Suppl. Fig. 2 Overview of the expert values for Cd (U) in round 2–4

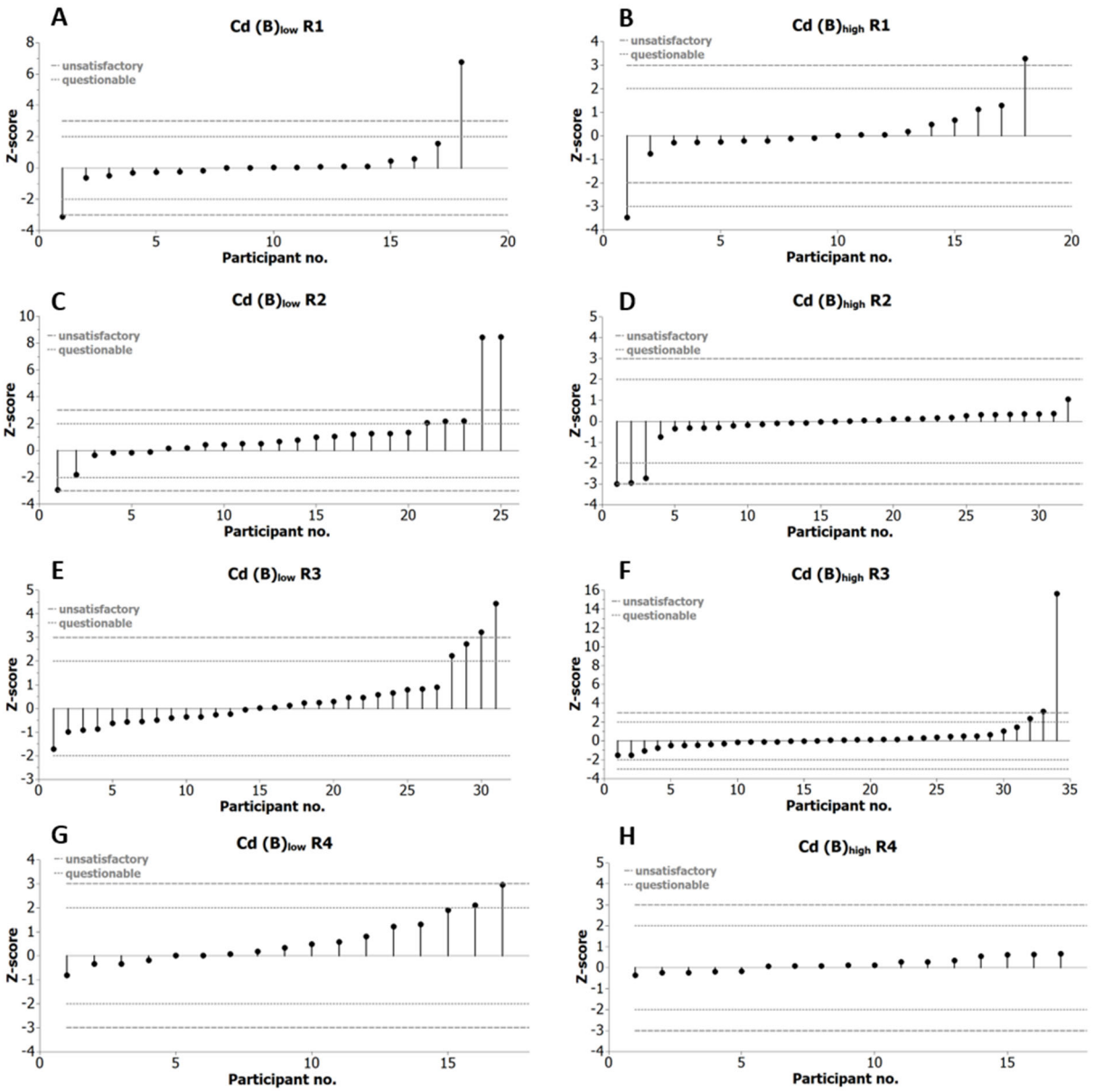


Suppl. Fig. 3 Overview of the expert values for Cd (B) in round 2–4



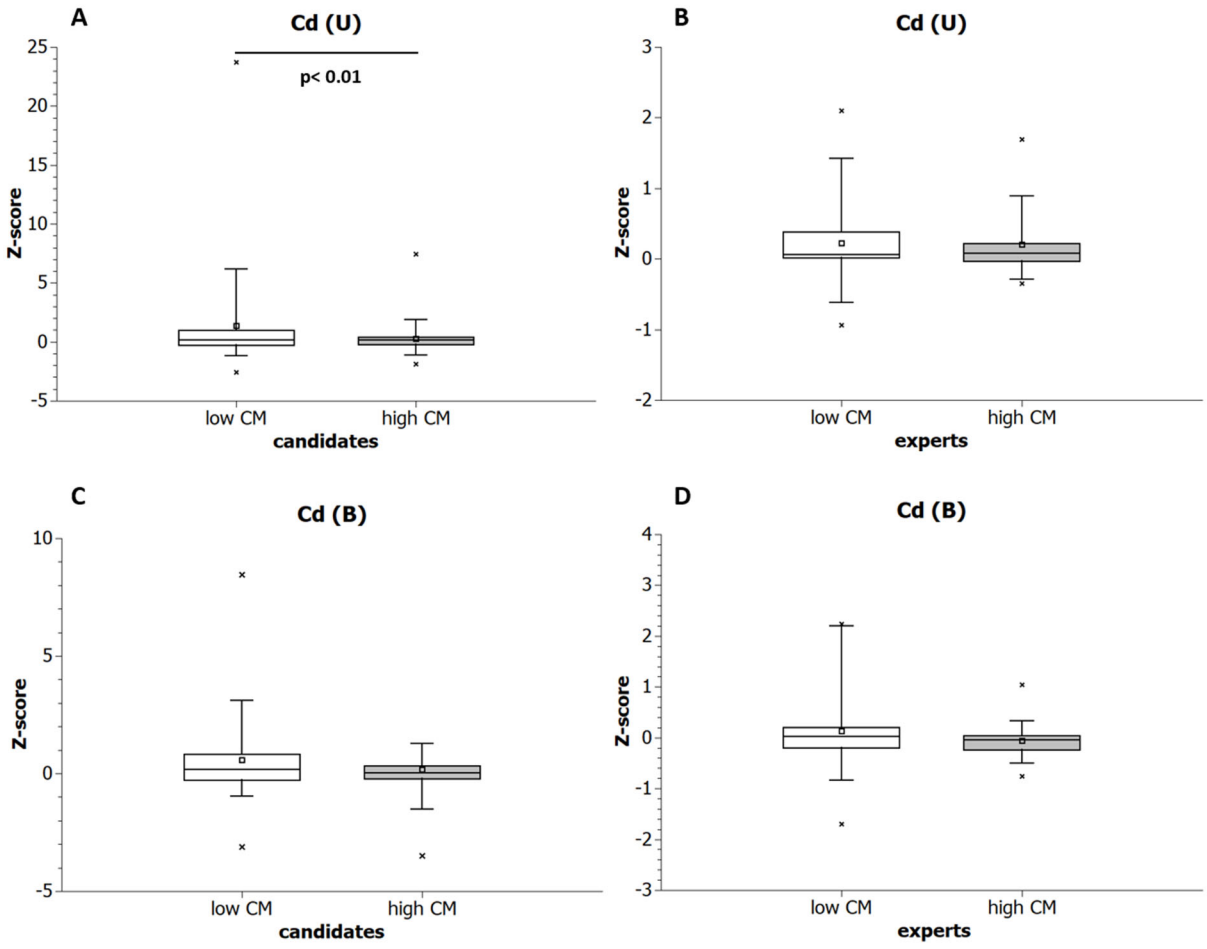
Suppl. Fig. 4 Z-scores of the participants` results in the ICI/EQUAS rounds 1-4 for Cd (U)

The Z-scores of the 20 participants with quantitative results in the 1st round were quite similar for Cd $(U)_{low}$ (A) and Cd $(U)_{high}$ (B) with one questionable and one unsatisfactory Z-score in each CM. In the low CM of the 2nd ICI/EQUAS (C), the participants reached with one Z-score ≥ 2.000 and ten Z-scores ≥ 3.000 the highest number of non-satisfying results of all rounds. From all high CMs, the outcome in the 2nd round (D) was worst as it resulted in three unsatisfactory Z-scores. The overall evaluation of the 3rd round was similar to the 1st round concerning the number of questionable and unsatisfactory Z-scores for the low (E) and the high CM (F). In the 4th round, the outcome for Cd $(U)_{low}$ (G) was comparable to round 1, while for Cd $(U)_{high}$ (H) only one participant obtained an unsatisfactory Z-score.

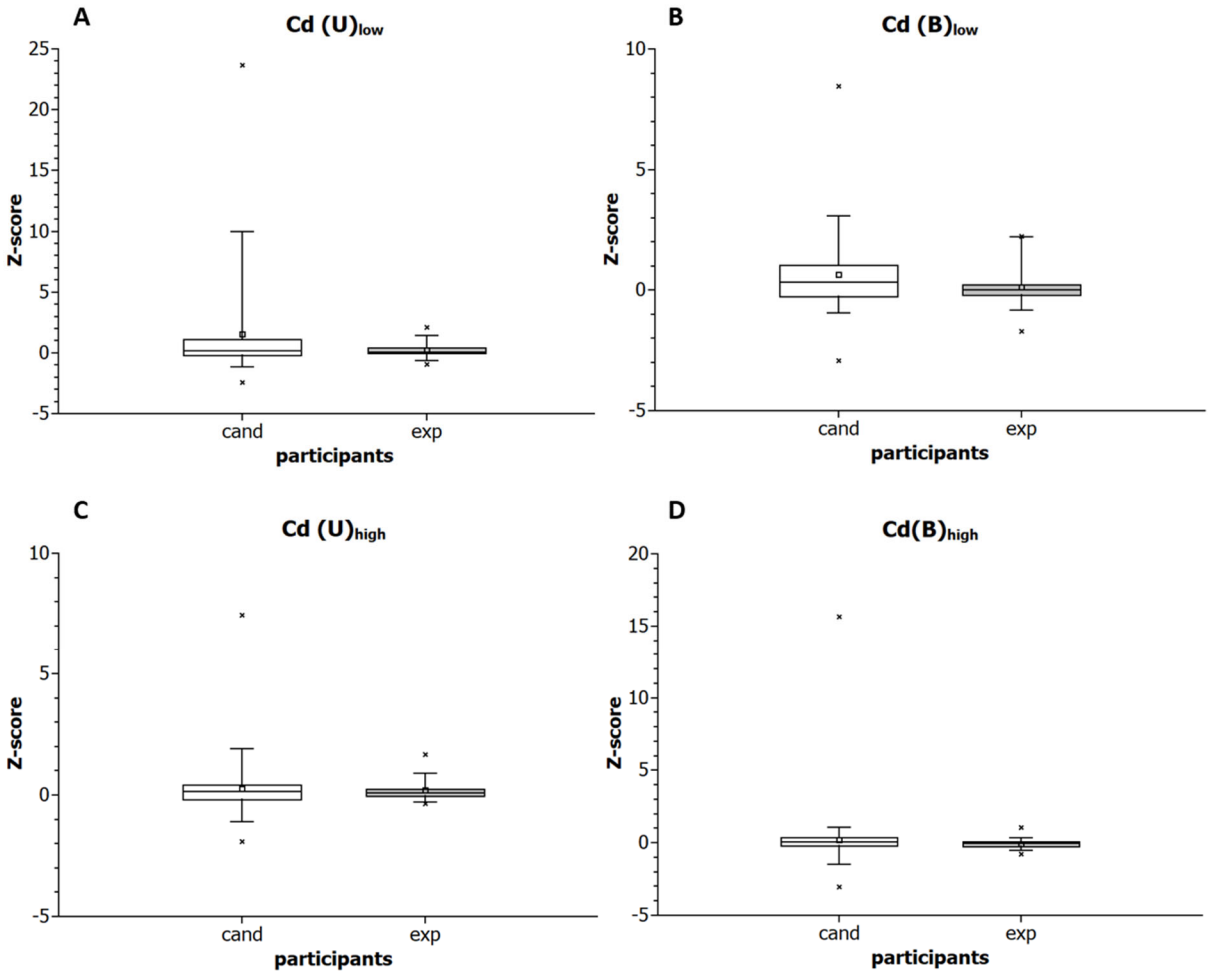


Suppl. Fig. 5 Z-scores of the participants`results in the ICI/EQUAS rounds 1-4 for Cd (B)

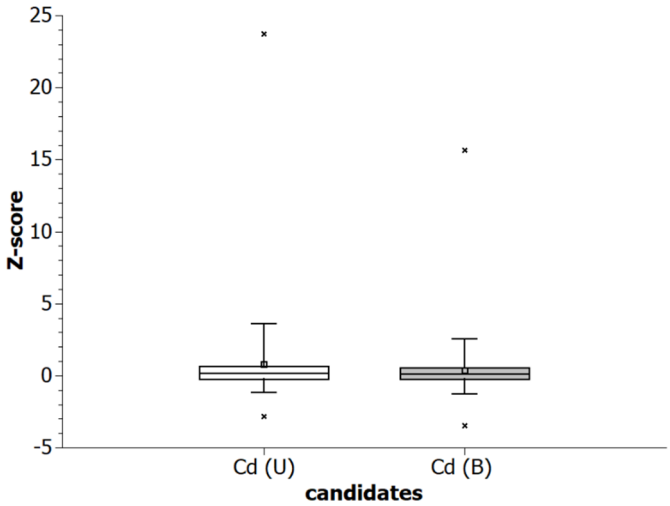
The Z-scores of the 18 participants with quantitative results in the 1st round were quite similar for Cd $(B)_{low}$ (A) and Cd $(B)_{high}$ (B) with two unsatisfactory Z-scores in each CM. In the low CM of the 2nd ICI/EQUAS (C), the participants reached with 4 Z-scores $\geq$ 2.000 and 7 Z-scores $\geq$ 3.000 the highest number of non-satisfying results of all rounds. From all high CMs, the outcome in the 2nd round (D) was worst as it resulted in one questionable and three unsatisfactory Z-scores. In the 3rd round, two questionable results and four unsatisfactory results were obtained by the participants for Cd $(B)_{low}$ (E). For Cd $(B)_{high}$ (F), one questionable and one unsatisfactory Z-score less were achieved. In the 4th round, there were no unsatisfactory results and two questionable results for the low CM (G). The analysis of the high CM in the 4th round resulted in satisfactory Z-scores for all participants with quantitative results (H).



Suppl. Fig. 6 Comparison of Z-scores for all low versus all high samples from candidates (round 1–4) and experts (round 2–4) for Cd (U) and Cd (B)



Suppl. Fig. 7 Comparison of Z-scores from candidates and experts for all Cd (U) and Cd (B) EQUAS samples (round 2–4)



Suppl. Fig. 8 Comparison of Z-scores from candidates for all Cd (U) and Cd (B) samples