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CERTIFICATE

1014-CM-60010-12

CERTIFIED REFERENCE MATERIALS CZ 60010

Lyophilised Human Urine

(o-cresol, hippuric acid, phenol and creatinine)

Date of issue: 6. April 2007

Valid until: 6. April 2012

Date of recertification 29. March 2012

Valid until: 6. April 2017

Presumed usage: Certified reference material is intended for quality control of the determination of creatinine, o-cresol, hippuric acid and phenol. O-cresol and hippuric acid (metabolites of toluene) are used for the biological monitoring of occupational exposure of toluene. Phenol, a metabolite of benzene, is used for biological monitoring of exposure to benzene, but phenol can also be used as an indicator of exposure to phenol as well.

The hippuric acid in concentrations under 750 $mg.l^{-1}$ is a natural compound of human urine.

Subcontractor: National Institute of Public Health, Šrobárova 48, 100 42, Praha 10-Vinohrady, Czech Republic

Responsible person: RNDr. Ilona Šperlingová, CSc

in compliance with the Czech Metrology Institute Methodical procedure No. 114-MP-C101. Preparation and certification of reference materials, and ISO REMCO Guides 34, 35.

CERTIFIED VALUES AND THEIR UNCERTAINTIES*

analyte	Certified value and uncertainty	Certified value and uncertainty
phenol	6,13 ± 0,63 $mg.l^{-1}$	65,1 ± 6,7 $\mu mol.l^{-1}$
o-cresol	1,14 ± 0,11 $mg.l^{-1}$	10,5 ± 1,0 $\mu mol.l^{-1}$
kyselina hippurová	1334 ± 63 $mg.l^{-1}$	7,44 ± 0,35 $mmol.l^{-1}$
creatinine	0,83 ± 0,18 $g.l^{-1}$	7,34 ± 1,60 $mmol.l^{-1}$

*expanded combined uncertainty, coefficient of expansion c = 2

Responsible person - CORM:

Ing. Jan Beránek

Český metrologický institut

Director of Regional branch, Prague:

Ing. Vladimír Peršl

Description and preparation:

Pooled human urine obtained from healthy persons occupationally exposed to toluene and those taking part in an inhalation experiment, stabilized by addition of 1 $g.l^{-1}$ sodium merthiolate (sodium ethylmercuri salicylate). Aliquots of urine (12.00 ml) were dispensed into 20-ml glass vials using an automatic Nichiryo Macromaster, freeze-dried, and the vials were hermetically stoppered. Accuracy of pipeting, expressed as the relative deviation of the mean was 0,16% a was determined by weighting.

Reconstitution of freeze-dried material:

Freeze-dried material is reconstituted for analysis by adding 12,00 ml bidistilled water at 20 °C - 25 °C and then by agitating this with a laboratory roller mixer at a frequency $1s^{-1}$ for 10 minutes. So obtained clear solution can be kept before use at 4 °C – 6 °C and with stopper tightly replaced for a period not exceeding fourteen days.

Homogeneity:

Creatinine, as well as the other analytes, is a soluble component of the urine. Nevertheless, the homogeneity of the creatinine has been tested. The testing was performed with six replicate determinations of the creatinine and hippuric concentrations by HPLC methods on ten units (every 100th from the whole batch – not randomly chosen so as to avoid trend inhomogeneity). Homogeneity testing was carried out by determining the phenol and o-cresol concentrations in fifteen units of reference material (every 75th from the whole batch). Three replicate analyses from each unit were performed using GC-FID method. No significant between-unit differences were found for the concentrations of all of the analytes mentioned above considering the analytical uncertainty and the significance level used α=0,05.

Stability:

Certified values are expected to be stable for the whole validity period stated of five years (provided the given storage, transport and handling conditions at 4 °C – 6 °C in dark). The stability will be continuously monitored over the whole validity period and registered users would be informed immediately on possible consequent changes.

Certified values and their uncertainties:

Certified values and their uncertainties were evaluated from the results of interlaboratory comparison using one analysis ANOVA and interactive program IPECA1. The certified values are the unweighted averages off accepted results , and their uncertainties are expanded combined uncertainties evaluated from the standard uncertainties of the interlaboratory comparison, homogeneity stability tests and from uncertainty of reconstitution (expansion coefficient k = 2).

Two or more independent methods were used in some laboratories.

Traceability:

SRM© 914a Creatinine Clinical Standard NIST (concentration of creatinine and its standard uncertainty: 99,7 ng/µl ± 0,3%), Phenol No.77610, lot 421187/1 61703052 99.5% (w/w)(GC), o-Cresol No.60990, lot 14781/1 14302004 99.5% (w/w)(GC) Fluka Co a hippuric acid (N-benzoylaminoacetic acid) >99%(w/w) Sigma-Aldrich Co. No H-6375 lot 30K34723) were used for calibration for traceability purposes.

Packing:

Three labelled 20 ml glass bottles, sealed with a rubber stopper and metal lid, plumbing, packed in a cardboard box, containing one copy of the Certificate.

Precaution:

The reference material was prepared from human urine of healthy persons, though it has to be considered as potentially infections and should be treated with appropriate care.

Certification analyses:

1. NIPH, Group for the assessment of occupational exposure to chemicals (RNDr. Ilona Šperlingová)
2. NIPH, Group for the assessment of occupational exposure to chemicals (ing. Slavka Grohová)
3. NIPH, Group for Chemical safety (RNDr. Marian Rucki).
4. NIPH Centre of environmental health (RNDr. Bohuslav Beneš)
5. IPH, Hradec Králové, affiliate Náchod (Ing. Irena Vančáková, Ing. Lhotský) Denisovo nábřeží 840, 547 01 Náchod
6. IPH, Liberec (Ing. Ludmila Stillerová, RNDr. Jiří Gracias), U Sila 1139, 463 11 Liberec 30
7. Faculty of natural sciences UK, (Prof. RNDr.Věra Pacáková), Albertov 2030, 120 00 Praha 2
8. Laboratorio di Sanità Pubblica, (Dr. Andrea Perico) ASL Firenze, ASL Firenze, Via di S.Salvi 12, 50 135 Firenze, Italy

Literature:

1. Kučera J, Faltejsek J: Interactive Programme for Evaluation of Circular Analyses, Fresenius J Anal Chem 352: 80-86 (1995)