Ural Scientific Research Institute for Metrology,
ROSSTANDART, RUSSIA

Report of CCQM-K149

Nitrogen mass fraction measurements in milk powder

Coordinating laboratory:

Ural Scientific Research Institute for Metrology, ROSSTANDART,

Ekaterinburg, **UNIIM**

Maria Medvedevskikh, Maria Krasheninina

With contributions from:

CENAM, Mexico: Mariana Arce Osuna, Diana Maria Morales Moreno

INMC, Colombia: Ivonne Alejandra González Cárdenas, Laura Vanessa Morales Erazo, Diego Alejandro Ahumada Forigua

Inmetro, Brazil: Eliane C. P. do Rego, Wagner Wollinger, Tânia M. Monteiro, Lucas J. de Carvalho

INACAL, Perú: Steve Ali Acco Garcia

GLHK, Hong Kong: Kelly WY Chan, Dr. Wai-hong Fung

RISE, Sweden: Conny Haraldsson

UMTS, Ukraine: Vladimir Gavrilkin, Sergey Kulik

INTI, Argentina: Lic. Gabriela Rodriguez

KazInMetr, Kazakhstan: Zharkynbekova Aida

Ekaterinburg
2018

Table of content

1 ABSTRACT....3
2 INTRODUTION....4
3 LIST OF PARTICIPANTS....5
4 SAMPLE....6
5 INSTRUCTIONS TO PARTICIPANTS....10
6 METHODS OF MEASUREMENT....10
7 RESULTS AND DISCUSSION....13
7.1 Uncertainty....13
7.2 Formulas....15
7.3 Nitrogen mass fraction in milk powder....16
7.4 Moisture measurement....20
7.5 Discussion....20
8 EQUIVALENCE STATEMENTS....20
9 CONCLUSIONS....21
10 ACKNOWLEDGEMENTS....21
11 REFERENCES....21
Appendix A – Technical Protocol....22
Technical protocol....22

1 ABSTRACT

The mass fraction of nitrogen in foodstuff is a very important parameter because the results of these measurements are often used for determination of protein mass fraction that is an important indicator of the quality of most food products and raw materials, for example dry milk powder.

The aim of this key comparison CCQM-K149 is to support National Measurement Institutes (NMIs) and Designated Institutes (DIs) to demonstrate the validity of the procedures the employed for determination of nitrogen mass fraction in milk powder, wheat powder, grain, egg powder, feed-stuff, biofuels, foodstuff and other.

The material, milk powder, used for this key comparison and pilot study has been selected to be representative of the food products. Furthermore, the material shows good properties regarding homogeneity and stability. The results from the key comparison and other experiments indicates that the milk powder studied is suitable for use as a certified reference material.

Ural Scientific Research Institute for Metrology (UNIIM) acted as the coordinating laboratory of this key comparison.

Eight NMIs and two DIs participated in this key comparison. This report contains the results of the key comparison.

2 INTRODUTION

Nitrogen mass fraction is a relevant quality indicator for food products and food raw materials. For the determination of this parameter Kjedahl Titrimetric method is often used. Despite the occurrence of a number of other methods for the determination of nitrogen content, such as Dumas method, infrared spectroscopy, chromatography etc., the Kjeldahl method continues to be the most used method in food analysis.

The Kjeldahl method is recommended as the reference method by various organizations, the most known of them are listed below [1]:

- AOAC International
- American Oil Chemists' Society
- American Public Health Association (APHA)
- American Society for Testing and Materials (ASTM)
- Association of American Cereal Chemists
- European Commission
- International Dairy Federation (IDF)
- International Organization for Standardization (ISO)
- U.S. Department of Agriculture

  U. S. Environmental Protection Agency (EPA)

But according to technical report participants are allowed to use any suitable methods of analysis.

China NIM (National Institute of Metrology) has calibration and measurement capabilities in determination of nitrogen mass fraction in non fat milk powder. Mechanism for measurement service delivery of this CMC is by CRM GBW08509. This CMC was approved on 13 June 2013 but a key comparison has never been carried out in the field of measurement.

3 LIST OF PARTICIPANTS

Eight NMIs and two DIs participated in the key comparison CCQM-K149. Table 1 contains the full names of all participating NMIs and DIs and contact persons.

Table 1 List of participants

№	NMI	Abbrev.	Country	Contact persons
1	National Institute of Metrology, Quality and Technology	INMETRO	Brazil	Eliane C. P. do Rego, Tânia M. Monteiro, Lucas J. de Carvalho, Bruno C. Garrido
2	Instituto Nacional de Metrología de Colombia	INMC	Colombia	Ivonne Alejandra González Cárdenas, Laura Vanessa Morales Erazo, Diego Alejandro Ahumada Forigua
3	Centro Nacional de Metrología	CENAM	Mexico	Mariana Arce Osuna, Diana Maria Morales Moreno
4	Instituto Nacional de Calidad	INACAL	Peru	Steve Ali Acco Garcia
5	Government Laboratory Hong Kong	GLHK	Hong Kong, China	Kelly WY Chan, Dr. Wai-hong Fung
6	Research Institute of Sweden	RISE	Sweden	Conny Haraldsson
7	National Institute of Industrial Technology	INTI	Argentina	Lic. Gabriela Rodriguez
8	State Enterprise All-Ukrainian State Research and production Center of Standardization, Metrology, Certification and Consumers' Rights Protection	UMTS	Ukraine	Vladimir Gavrilkin, Sergey Kulik
9	Ural Scientific Research Institute for Metrology	UNIIM	Russia	Maria Medvedevskikh, Maria Krasheninina
10	Republican State Enterprise "Kazakhstan Institute of Metrology" Committee for technical regulation and metrology Kazakhstan Investments and Development Ministry	KazInMetr	Kazahstan	Zharkynbekova Aida

4 SAMPLE

The comparison material for the CCQM-K149 was dry milk powder.

Dry milk powder was preliminary homogenized with the help of grinding machine. Material was divided into several fractions, nitrogen mass fraction was measured in each fraction in 5 parallels. It was shown that fraction with partials from 200 to 300 μm has the least standard deviation in nitrogen mass fraction measurements. After the process of homogenization the material of dry milk powder was subdivided into double plastic bags. Each sample contained 100 g.

After preparation of the samples, homogeneity test has been carried out. Homogeneity test for milk powder is presented in Table 2.

Table 2 Results of homogeneity testing between bottles (5 replicates for each bottle)

Vial	Nitrogen mass fraction, %					Mean value in vial, %
	1	2	3	4	5	
1	2,91	2,87	2,92	2,89	2,84	2,89
2	2,90	2,86	2,90	2,85	2,88	2,88
3	2,90	2,90	2,90	2,89	2,92	2,90
4	2,86	2,85	2,81	2,85	2,87	2,85
5	2,85	2,89	2,90	2,84	2,84	2,87
6	2,92	2,86	2,88	2,89	2,88	2,89

In order to estimate the inhomogeneity contribution u_h, a 1-way Analysis of Variances (ANOVA) has been carried out with the experimental homogeneity data (table 1). The standard uncertainty due to (in)homogeneity, u_h, value for milk powder (see Table 3, 4) was calculated according to ISO Guide 35 using the Equations (1) and (2).

$$u_h = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \quad (1)$$

$$u_h = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{N(\mathrm{n}-1)}}, \quad (2)$$

where N=6, n=5.

Table 3 ANOVA analysis

Vial	***number***	***sum***	***average***	***dispersion***
1	5	14,43	2,886	0,00103
2	5	14,39	2,878	0,00052
3	5	14,51	2,902	0,00012
4	5	14,24	2,848	0,00052
5	5	14,32	2,864	0,00083
6	5	14,43	2,886	0,00048

Table 4 ANOVA analysis

source	***SS***	***df***	***MS***	***F***
Among	0,0090	5	0,001797	3,08114
Within	0,014	24	0,000583	
Sum	0,023	29		
standard uncertainties due to inhomogeneity, u_h		**0,015**	%	Equation (1)
standard uncertainties due to inhomogeneity, $\boldsymbol{u_h}$		**-**	**-**	Equation (2)
relative standard uncertainties due to inhomogeneity, $\boldsymbol{u_{ho}}$		**0,53**	**%**	

Stability test for milk powder is presented in Table 5 and Figure 1. Long-term stability study has been conducted with the help of classical experiment. Six samples were used.

Table 5 Results of measurement of nitrogen mass fraction in milk powder

№	Month	Nitrogen mass fraction, %
1	1	2,87
2	2	2,89
3	3	2,86
4	4	2,89
5	5	2,87
6	6	2,88
mean of stability test, Xs		2,88
standard deviation of the data of key comparison participants, S		0,012
Xs+S		2,89
Xs-S		2,87
slope,*b*		0,00007
standard uncertainty of slope, u_{slope}		0,0071
confidence interval $t_{0,05;(n-2)} \cdot u_{slope}$		0,0072
standard uncertainty due to long-term (in)stability, u_s		0,017
relative standard uncertainty due to long-term (in)stability, u_{so}, %		0,58
time measurements in key comparison, t, days (according to isochronous experiment)		180

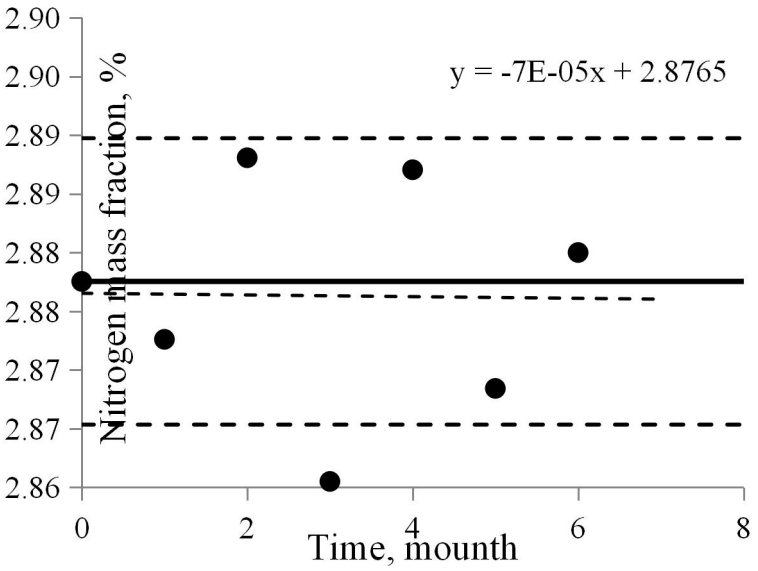

Figure.1 - Stability test for milk powder

Data in Table 5 were evaluated using linear regression method. Standard uncertainty due to instability was calculated using formula:

$$u_s = \sqrt{u_{slope} \cdot t}\,, \tag{3}$$

Additionally, standard uncertainty due to short-term instability has been estimated. The statistical evaluation of the homogeneity, long-term and short-term stability has indicated that standard uncertainties due to inhomogeneity is 0,015 %, and long-term instability is 0,017 % and short-term instability is 0,008 %.

The samples have been sent to the participants by DHL on 25th August 2017. Each sample has been accompanied by veterinary certificate of international view. All samples arrived to their destination without damage but in different countries, it has taken different time: from several days to two months. The dispatch dates and receipt dates are given in Table 6.

The deadline for reporting results was set by end of February 2018 in order to prepare a presentation for discussion at the CCQM IAWG meeting in April 2018. All participants reported their results in time (except KazInMetr).

Table 6 Sample sent dates, receipt dates and report dates

№	NMI	Abbrev.	Country	Date of receipt the sample	Report sending information
1	National Institute of Metrology, Quality and Technology	INMETRO	Brazil	30/09/2017	In time
2	Instituto Nacional de Metrología de Colombia	INMC	Colombia	30/08/2017	In time
3	Centro Nacional de Metrología	CENAM	Mexico	31/08/2017	In time
4	Instituto Nacional de Calidad	INACAL	Peru	30/09/2017	In time
5	Government Laboratory Hong Kong	GLHK	Hong Kong, China	30/08/2017	In time
6	Research Institute of Sweden	RISE	Sweden	29/08/2017	Later
7	National Institute of Industrial Technology	INTI	Argentina	30/08/2017	In time
8	State Enterprise All-Ukrainian State Research and production Center of Standardization, Metrology, Certification and Consumers' Rights Protection	UMTS	Ukraine	30/08/2017	In time
9	Ural Scientific Research Institute for Metrology	UNIIM	Russia	-	In time
10	Republican State Enterprise "Kazakhstan Institute of Metrology" Committee for technical regulation and metrology Kazakhstan Investments and Development Ministry	KazInMetr	Kazahstan	28/08/2017	Report was sent only13/04/18

5 INSTRUCTIONS TO PARTICIPANTS

Technical protocol has been sent to the participants by e-mail.

The technical protocol (appendix A) contained background information, timing of the comparison, and information on the participating institutes. Information on sample preparation and recommendation of condition for measurements was given.

Each participant is allowed to use any suitable method of analysis.

Participants were requested to report the results of nitrogen mass fraction in milk powder. The results should be reported accompanied by a full uncertainty statement (including a combined standard uncertainty and an expanded uncertainty with a coverage factor applied). In addition, the report should include technical details on the measurement procedure, traceability links (as calibrations) and uncertainty contributions.

Measurement results (mass fraction of nitrogen in milk powder) should be corrected in terms of dry-matter.

6 METHODS OF MEASUREMENT

Nine participants used Kjeldahl method for the measurements and one participant used method of elemental analysis. Some details on measurements as derived from the reports are given in Table 7 and Table 8. Participant from Colombia presented measurement results obtained with Kjeldahl method for the key comparison and an information result by ion chromatography (IC) with conductivity detection.

Table 7 Details of sample mass and titrant

Country	NMI/DIS	Method of analysis	Approx. sample mass	Additional information
Colombia	INMC	Kjeldahl	(0,40 - 0,41) g	Titrant: Hydrochloric acid, 0,1 M
Colombia	INMC	Ion chromate-graphy (IC) with conductivity detection	(0,40 - 0,41) g	After the pre-treatment the sample is diluted gravimetrically to perform the determination of ammonium by capillary ion chromatography using external calibration method with internal standard
Mexico	CENAM	Kjeldahl	1 g	Titrant: Hydrochloric acid, 0,1 M
Brazil	Inmetro	Kjeldahl	0,5 g	Titrant: Sulphuric acid, 0,25 M
Russia	UNIIM	Kjeldahl	1 g	Titrant: Sulphuric acid, 0,05 M
Peru	INACAL	Kjeldahl	(0,995 - 1,005) g	Titrant: Hydrochloric acid, 1 M
Hong Kong	GLHK	Kjeldahl	1 g	Titrant: Hydrochloric acid, 1 M
Sweden	RISE	Elemental analyzer	200 mg	LECO CHN 628

Continuation of Table 7

Country	NMI/ DIS	Method of analysis	Approx. sample mass	Additional information
Ukraine	UMTS	Kjeldahl	(0,50 - 0,85) g	Titrant: Sulphuric acid, 0,0506 mol•l-1
Argentina	INTI	Kjeldahl	(0,45 - 0,55) g	Titrant: Hydrochloric acid, 1 M
Kazakh-stan	KazIn Metr	Kjeldahl		

Table 8 Traceability details

Institute	Country	Traceability
CENAM	Mexico	Traceable to DMR-74h Hydrochloric acid (0.09989 N).
INMC	Colombia	Traceable to the reference material A02 potassium hydrogen phthalate, produced by the Slovak Institute of Metrology.
INMETRO	Brazil	Traceable to Sodium Carbonate and Standard Reference Material NIST SRM 1849a
INACAL	Perú	Traceable to Potassium Hydrogen Phthalate that is certified by coulometric titration
GLHK	Hong Kong	Traceable to NMIJ 3005-a Sodium carbonate
RISE	Sweden	Traceable to TRIS reference material from Slovak Institute of Metrology LOT A0704414
UMTS	Ukraine	Certified reference material of Sodium carbonate NIOCHIM (DSZU 023.36-06); mass fraction of Sodium carbonate 99.668
INTI	Argentina	Traceability of values used for calibration: -Hydrochloric acid, 0,1 M (f=1) TitriPUR, Batch HC55155760. The concentration of this volumetric solution was determined with volumetric standard TRIS (Merck). The determined titer at 20°C was 1,000 with an expanded measurement uncertainty of ±0,003 (k=2 coverage factor for 95% coverage probability). The certified value is traceable to primary standard NIST SRM 723e by means of volumetric standard TRIS, measured in the accreditated calibration laboratory of Merck KGaA in accordance to DIN EN ISO/IEC 17025. -L-Tryptophan, Merck, assay (perchloric acid titration, calculated on dry substance) > 99,0%. -Ammonium sulfate, Merck, assay (alkalimetric) > 99,5%. -L-Lysine mono-hydrochloride (C6H15ClN2O2), assay 99,9% (mass fraction). -Standard Reference Material 1849 a, NIST, Infant/Adult Nutritional Formula.
KazInMetr	Kazakhstan	Traceble to UNIIM GSO 10450-2014 (high purity sodium carbonate that is used for determination molar concentration of sulphuric acid) that is certified by coulometric titration
UNIIM	Russia	Traceble to UNIIM GSO 10450-2014 (high purity sodium carbonate that is used for determination molar concentration of sulphuric acid) that is certified by coulometric titration

7 RESULTS AND DISCUSSION

7.1 Uncertainty

Participants have used different approaches for estimations of measurement uncertainty of nitrogen mass fraction by Kjeldahl method and Elemental method of analysis and have accounted different sources of uncertainty in budget of uncertainty. Some details about sources of uncertainty are given in Table 9.

Table 9 Details about results and sources of uncertainty

Institute	Accounted sources of uncertainty
CENAM	hydrochloric acid concentration HCl mass for the end point of the sample titration. HCl mass for the end point of the blank titration. dry mass of the sample.
INMC	**Type A** repeatability of measurement results **Type B** mass of titration (blank and test sample) concentration of hydrochloric acid standard sample mass moisture molecular weight of nitrogen detection of end point of titration
INMETRO	repeatability of the measurements volume of the sulfuric acid solution used in the titration of the sample volume of the sulfuric acid solution used in the titration of the blank mass of sample titrated sodium carbonate mass used in the standardization of the acid volume of the sulfuric acid solution used in the titration of the Na_2CO_3 for standardization certified purity of the sodium carbonate molar mass of nitrogen molar mass of the sodium carbonate moisture mass fraction determined in the sample
INACAL	**Type A** - repeatability of measurement results **Type B** - standard uncertainty due to molecular weight of nitrogen and Potassium Hydrogen Phthalate
GLHK	**Type A** - repeatability of measurement results. **Type B** According to GUM. No additional information was given.
RISE	**Type A** - mean instrument signal for test portion 1 - mean instrument signal for Reference for test portion 1 - mean instrument signal for test portion 2 - mean instrument signal for Reference for test portion 2 - mean instrument signal for test portion 3 - mean instrument signal for Reference for test portion 3 **Type B**

	- amount content of base expressed as TRIS - atomic weight of nitrogen

Continuation of Table 9

Institute	Accounted sources of uncertainty
UMTS	**Type A** - repeatability of measurement results **Type B** - sample weighting - EP determination - titrant volume determination - titrant concentration determination - nitrogen atomic mass uncertainty Uncertainty of the moisture determination result also has been taken into account
INTI	**Type A** - repeatability of measurement resultsType **Type B** - standard uncertainty due to Sample weight - standard uncertainty due to Titrant volume of hydrochloric acid standard volumetric solution (Blank and test sample) - standard uncertainty due to Concentration of hydrochloric acid standard volumetric solution
KazInMetr	**Type A** - repeatability of measurement results **Type B** - standard uncertainty due to sample weight - standard uncertainty due to titrant volume of sulphuric acid standard volumetric solution (Blank and test sample) - standard uncertainty due to Concentration of sulphuric acid standard volumetric solution - standard uncertainty due to atomic weight of nitrogen - standard uncertainty due to moisture measurements
UNIIM	**Type A** - repeatability of measurement results **Type B** - standard uncertainty due to sample weight - standard uncertainty due to titrant volume of sulphuric acid standard volumetric solution (Blank and test sample) - standard uncertainty due to Concentration of sulphuric acid standard volumetric solution - standard uncertainty due to certified value of GSO 10450-2014/ that was used for determination of molar concentration of sulphuric acid - standard uncertainty due to detection of end point of titration

7.2 Formulae

Preliminary inspection of value x_i and associated uncertainties $u(\overline{x}_i)$ has been carried out in accordance with CCQM guidance note [3] using the following equation

$$\frac{x_i - med(x)}{u(x_i)}, \tag{4}$$

The results of preliminary inspection have shown that in general there are consistent results with a small number of outlying results. It means that it's case – C according to the CCQM guidance.

Checks of consistency have performed according to the CCQM guidance note [3] using the algorithm shown below (Measurement result from Kazakhstan is extremely high that is why it is not taken into account).

$$\overline{x}_u = \sum_{i=1}^{m} \frac{x_i / u^2(x_i)}{\sum_{i=1}^{m} 1/u^2(x_i)}, \tag{5}$$

$$\chi^2_{obs} = \sum_{i=1}^{m} \left(\frac{x_i - \overline{x}_u}{u(x_i)} \right)^2, \tag{6}$$

where x_i - result of value of *i* NMI, $u(\overline{x})$ - standard uncertainty of $\overline{x}$.

After calculations using formulas (4), (5) was compared χ^2_{obs} with m-1 and with $\chi^2_{0.05,m-1}$, the 95 percentile of χ^2 with m-1 of freedom.

If $\chi^2_{obs} < m-1$, it is normally safe to proceed with the assumption that the results are mutually consistent and that the uncertainties account fully for the observed dispersion of values.

If $m-1 < \chi^2_{obs} < \chi^2_{0.05,m-1}$ the data provide no strong evidence that the reported uncertainties are inappropriate, but the remains a risk that additional factors are contributing to the dispersion. Refer to the prior working group decision on presumptive consistency and proceed accordingly.

If $\chi^2_{obs} > \chi^2_{0.05,m-1}$ the data should be considered mutually inconsistent.

Candidates of the key comparison reference values (KCRV) were estimated following the CCQM guidance note [3] using different approaches. The results from Kazakhstan has not been taken into account to determine the KCRV. Results and uncertainties have been taken from the reports as they were. Formulas for calculation are shown below.

$$\overline{x} = \frac{1}{m} \sum_{i=1}^{m} x_i, \tag{7}$$

$$u(\overline{x}) = \frac{\sum_{i=1}^{m} (x_i - \overline{x})^2}{m(m-1)}, \tag{8}$$

where x_i - result of value of *i* NMI, $u(\overline{x})$ - standard uncertainty of $\overline{x}$.

Uncertainty-weighted mean

$$\bar{x}_u = \sum_{i=1}^{m} w_i x_i \,, \tag{9}$$

$$w_i = \frac{1/u^2(x_i)}{\sum_{i=1}^{m} 1/u^2(x_i)} \,, \tag{10}$$

$$\frac{1}{u^2(\bar{x}_u)} = \sum_{i=1}^{m} 1/u^2(x_i) \,, \tag{11}$$

where $u(x_i)$ - standard uncertainty of x_i .

Median

$$med(x) = \begin{Bmatrix} \frac{1}{2}(x'_{m/2} + x'_{m/2+1}), & even\ \ m \\ x'_{(m+1)/2}, & m\ \ \ odd \end{Bmatrix}, \tag{12}$$

$$u^2(med(x)) = \frac{\pi}{2m}\hat{\sigma}^2 \,, \tag{13}$$

$$\hat{\sigma} = 1.483 med(|d_i|) \,, \tag{14}$$

where $d_i = x_i - med(x)$.

7.3 Nitrogen mass fraction in milk powder

The reported values of nitrogen mass fraction and uncertainties of all results have been summarized in Table 10. Estimations of candidates KCRV have been obtained by different approaches (arithmetic mean, weighted mean, median) are presented in Table 10. The same results are displayed graphically in Figures 2, 3.

It is proposed to use the median of the KCRV, because:

- $\chi^2_{obs} > \chi^2_{0.05,\mathrm{m}-1}$ in this case the data is mutually inconsistent,
- The reported uncertainties are not very different,
- There are two extreme values according to $(x_i - med(x))/u(x_i)$,
- According to Figure 2 transformed distribution for reported results of NMIs and DIs for nitrogen mass fraction is asymmetric.

Table 10 Reported values of nitrogen mass fraction and uncertainties

№	Kind of com-pari-son	NMI/ DIS	Nitrogen mass fraction, %	Combined standard uncertainty, u_c, %	Expanded uncertainty, U(k=2), %	di, %	U(di), %	Verdict
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1	Key	CENAM	2,82	0,005	0,012	-0,07	0,02	-
2	Key	INMC	2,83	0,035	0,070	-0,06	0,07	+
3	Key	Inmetro	2,84	0,025	0,050	-0,05	0,05	+
4	Key	UNIIM	2,87	0,012	0,024	-0,02	0,03	+
5	Key	INACAL	2,89	0,014	0,029	0,00	0,04	+
6	Key	GLHK	2,89	0,032	0,064	0,00	0,07	+
7	Key	RISE	2,90	0,006	0,011	0,01	0,02	+
8	Key	UMTS	2,90	0,017	0,034	0,01	0,04	+
9	Key	INTI	2,93	0,012	0,024	0,04	0,03	-
10	Key	Kazin-metr	3,43	0,16	0,31	0,55	0,32	-
median			**2,887**	**0,011**	**0,02**	**KCRV**		
mean			2,873	0,01	0,02			
weighted mean			2,862	0,00	0,01			
Consistency test						**Conclusion**		
χ^2_{obs}			$\chi^2_{0.05,m-1}$		m	$\chi^2_{obs} > \chi^2_{0.05,m-1}$		
159.21			15.5		9	**inconsistent**		

Note: Measurement result from Kazakhstan is not taken into account for calculation median, mean and weighted mean.

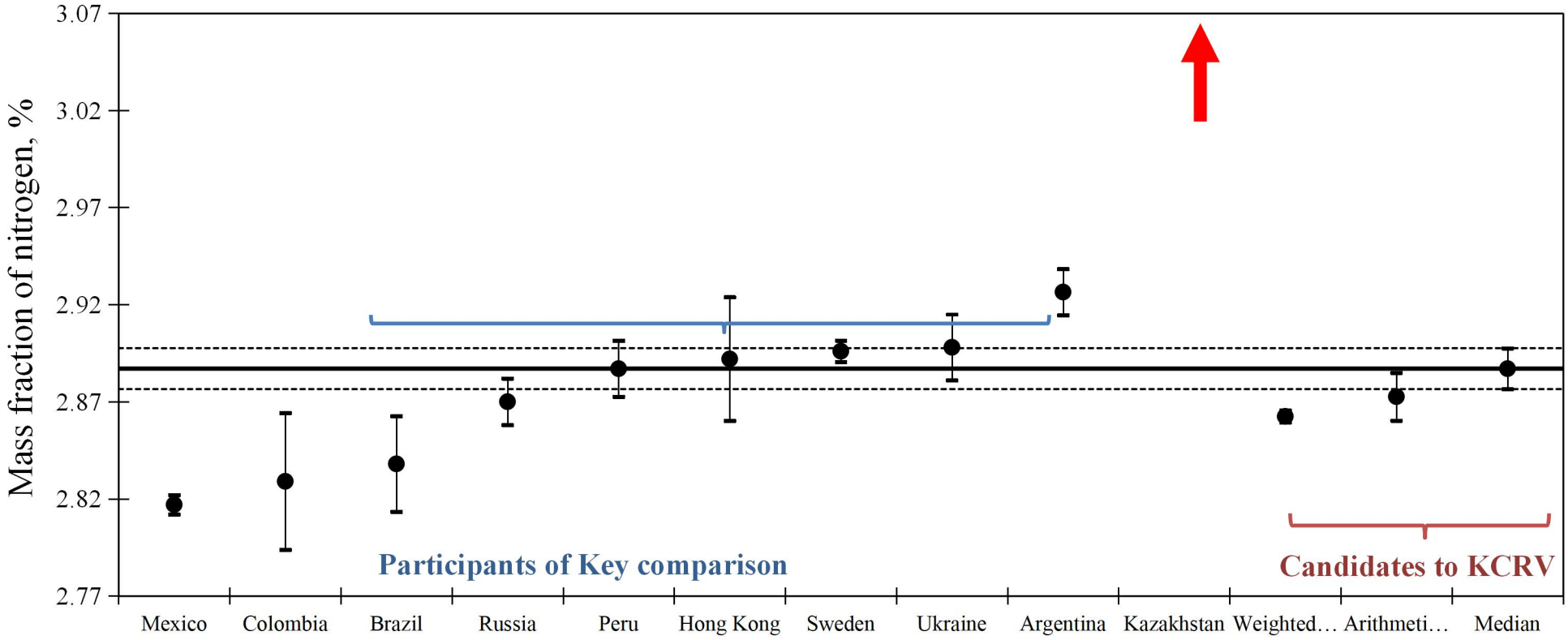

Figure 2 Error bars show standard uncertainty. The solid and dashed horizontal lines are the **median** and upper and low limits of the corresponding standard uncertainty, respectively.

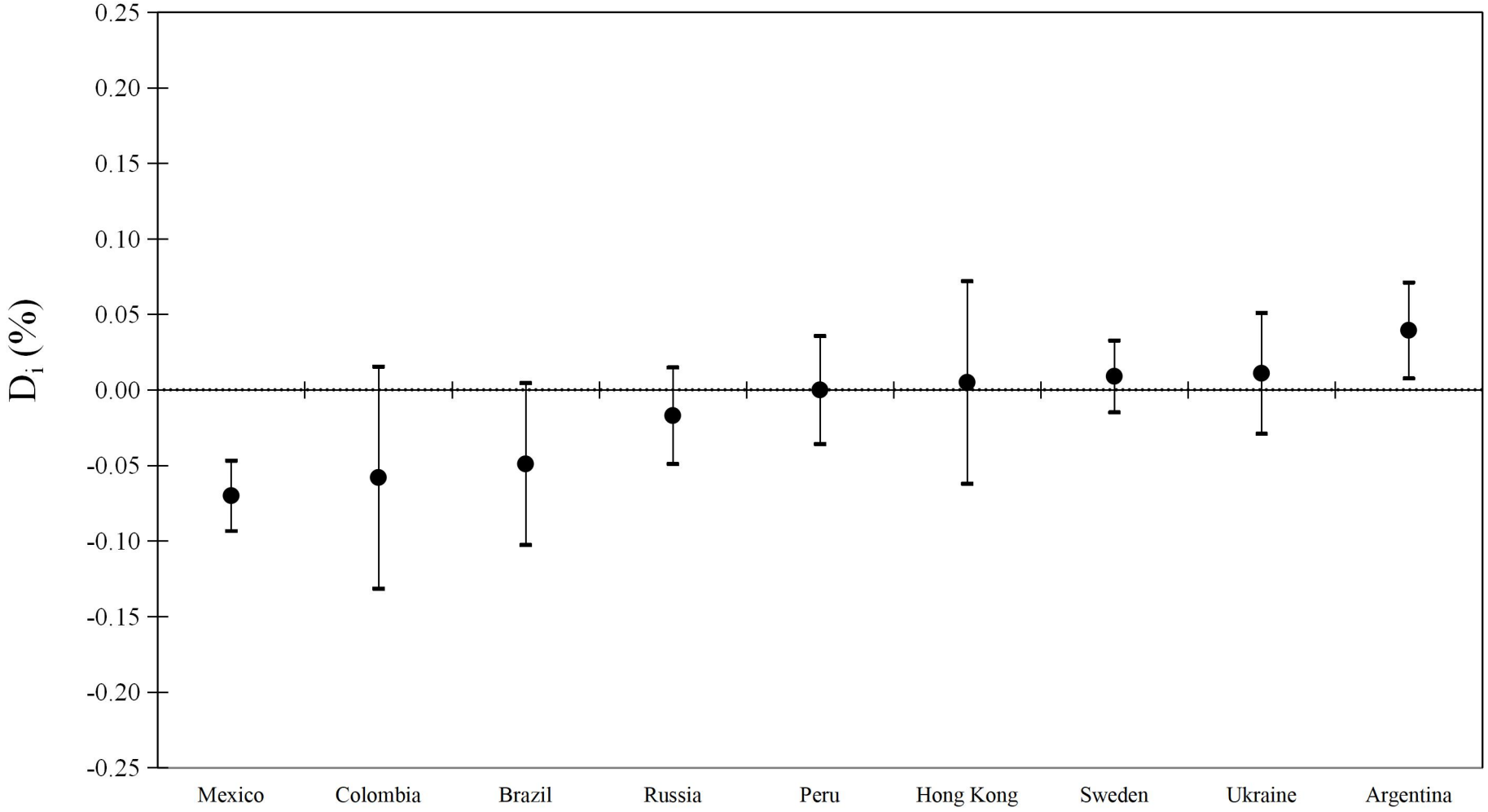

Figure 3 Degrees of equivalence d_i and expanded uncertainty $U（d_i）$(k=2)

7.4 Moisture measurement

In accordance with Technical protocol of CCQM-K149 each participant presented nitrogen mass fraction measurement in terms of dry matter. The following drying mode was offered in technical protocol: Temperature is 102±2 °C; Drying time is 2,5 hours; Time of cooling is 40 min.

Results of moisture mass fraction measurement is given in table 11.

Table 11 Results of moisture mass fraction measurement

№	Kind of com-pari-son	NMI/ DIS	Moisture mass fraction, %	Combined standard uncertainty, u_c, %	Expanded uncertainty, U(k=2), %
1	*2*	*3*	*4*	*5*	*6*
1	Key	CENAM	3,389	0,0055	0,011
2	Key	INMC	3,36	0,064	0,129
3	Key	Inmetro	4,154	0,0095	0,019
4	Key	UNIIM	3,41	0,075	0,15
5	Key	INACAL	3,343	0,007	0,014
6	Key	GLHK	3,0875	0,0102	0,0204
7	Key	RISE	3,36	0,04	0,08
8	Key	UMTS	3,508	0,112	0,224
9	Key	INTI	3,2083	0,07	0,14
10	Key	Kazinmetr	3,31	0,18	0,36

7.5 Discussion

Taking into account the final results it is possible to say that measurement results of almost all participants are consistent between each other.

8 EQUIVALENCE STATEMENTS

The equivalence statements have been calculated according to the BIPM guideline. The degree of equivalence (and its uncertainty) between a NMI result and the KCRV is calculated according to the following equations:

$$d_i = x_i - x_{ref}, \tag{15}$$

$$U(d_i) = 2\sqrt{\left(u^2(x_i) + u^2(x_{ref})\right)}, \tag{16}$$

where d_i is the degree of equivalence between the NMI result x_i and the KCRV x_{ref}, and $U(d_i)$ is the expanded uncertainty *(k = 2)* of the d_i calculated by combining the standard uncertainty $u(d_i)$ of the NMI result *xi* and the standard uncertainty $u\ x_{ref}$ of the KCRV x_{ref} (it is supposed that $\mathrm{cov}(x_i, x_{ref})$ is ineligible). The equivalence statements for CCQM-K149 are given in Table 10 and Figures 2, 3.

9 CONCLUSIONS

The median is proposed for the KCRV. The use of median value is agreed by all participants and the CCQM IAWG.

This key comparison can be used in order to support calibration and measurement capabilities in determination of nitrogen mass fraction in in milk powder, wheat powder, grain, egg powder, feed-stuff.

For elemental analyzers operating according to the Dumas principle, i.e. combustion of the sample followed by reduction of nitrogen compounds to N_2 that is selectively detected, organically bound nitrogen can be determined in any organic material of biological origin (e.g. biofuels, or foodstuff) and synthetic organic compounds.

10 ACKNOWLEDGEMENTS

UNIM gratefully acknowledges the help and collaboration from CENAM, INMC, INMETRO, INACAL, GLHK, RISE, UMTS, INTI, KAZINMETR.

11 REFERENCES

1. J.C. Moore, W.Vries, M. Lipp, J.C. Grifiths, D.R. Abernethy Total protein methods and their potential utility to reduce the risk of food protein adulteration *Compr. Rev. Food Sci. F*. **9(4)** 330–351 (2010).
2. ISO 9277 Determination of the specific surface area of solids by gas adsorption. BET method. International Organization for Standardization, Geneva (2010).
3. CCQM Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence. Version: 10.

Appendix A – Technical Protocol
CCQM-K149- Key comparisons

Nitrogen mass fraction measurements in dry milk powder
Technical protocol

1. Introduction

Mass fraction of nitrogen is very important pointer because the results of these measurements are often used for determination of protein mass fraction that is an important indicator of the quality of the vast majority of food products and raw materials, in particular dry milk powder. Proteins-enzymes catalyze chemical reactions; protein along with fats and carbohydrates is one of the indicators characterizing the energy value of food, so its definition is mandatory for all food products.

After discussing results of Pilot study in the field of nitrogen mass fraction in dry milk powder CCQM-P167 during the meeting in Daejeon in October 2016 it was agreed to conduct the Key comparison in this field of measurement.

The comparison is being carried out for the purpose of the confirmation of follow measurement capacity:

NMI Service Identifier	Measurement Service Category	Matrix	Measurand		Range of certified values in reference materials			Range of expanded uncertainty for certified values				Mechanism for measurement service delivery	Comments It is being recommended
			Analyte of component	Quality	From	To	Unit	From	To	Unit	Is the expanded uncertainty a relative one?		
	Other	Dry milk powder	nitrogen	Mass fraction	1	6	%	-	-				

2. Measurand and reporting

Mandatory measurand (for CCQM) – value of mass fraction of nitrogen in terms of absolute dry mass.

The aim of CCQM-K is to measure mass fraction of nitrogen in terms of absolute dry mass.

Each participant shall report the results for the values of mass fraction of nitrogen. The results should be reported in mass fractions, accompanied by a full uncertainty statement (including a combined standard uncertainty and an expanded uncertainty with a coverage factor applied). In addition the report should include technical details on the measurement procedure, traceability links and uncertainty contributions.

Mass fraction of moisture can be change during the transportation and storage time therefore, it's necessary to measure moisture mass fraction by each participant and present content of nitrogen as mass fraction of nitrogen in terms of absolute dry mass. The most convenient method for moisture mass fraction: Temperature is 102±2 °C; Drying time is 2,5 hours; Time of cooling is 40 min.

3. Guidance values and target uncertainty

Analyte / matrix: the objects of comparisons are nitrogen mass fraction and moisture mass fraction in milk powder.

Reference material (Standard Sample) of milk powder composition GSO 9563-2010 (RU Certificate of Standard Sample approval № 4389 dated 24.04.2015) is used as sample for current key comparison Sample of dry milk powder in the range nitrogen mass fraction from 1 % to 6 % and in the range of moisture from 3 % to 6 % is delivered by UNIIM..

Target uncertainty is expected on the level of 0,05 %.

4. KCRVs

Processing of obtained measurement results of nitrogen mass fraction and moisture mass fraction will be carried out according to the following articles and document:

- Cox M.G. "The evaluation of key comparison data" [2]
- Jorg W.Muller. "Possible Advantages of a Robust Evaluation of Comparisons" [3]

It's offered to try different approaches: the arithmetic mean, weighted mean, median for the evaluation of reference value. It will be done only for the information and will be excluded from the report B

- CCQM Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence. Version: 10

5. Methods of measurement

Each participant may use any suitable method(s) for the measurement of the mass fraction of nitrogen.

More realistic for this type of measurements seems to be the application of Titrimetric method of Kjedahl. Despite the occurrence of a number of the other methods for the measurements of nitrogen content, such as Dumas method, infrared spectroscopy, chromatography etc., Kjeldahl method remains the most accurate and reliable method of the measurement of nitrogen (protein) mass fraction. Kjeldahl method is admitted as a reference method by various organizations, the most known of them are listed on this below [4]:

- AOAC International
- American Oil Chemists' Society
- American Public Health Association (APHA)
- American Society for Testing and Materials (ASTM)
- Association of American Cereal Chemists
- European Commission
- International Dairy Federation (IDF)
- International Organization for Standardization (ISO)
- U.S. Department of Agriculture

  U. S. Environmental Protection Agency (EPA)

6. Planned time schedule

call for participants:	by end of April 2017
latest registration of participant:	by end of July 2017 (updated)
latest arrival of samples at participants:	by end of September 2017
latest report of results:	by end of February 2018
report A:	by end of May 2018
report B:	by end of July 2018

7. Samples

Sample of dry milk powder in the range nitrogen mass fraction from 1 % to 6 % and in the range of moisture from 3 % to 6 % is delivered by UNIIM.

Packaging and labeling: Sample of dry milk powder with mass 100 g is packed into special water-proof double polyethylene packages hermetically sealed with double seam with the size 150×150 mm. The package has the label with the sample name.

Storage conditions:

- Ambient temperature, °C 7±3
- Protection from the straight sun light

Storage life is 6 months.

Short-term stability was confirmed by storage of samples for 2 weeks under various conditions (air temperature was varied from + 5ºC to +40ºC, relative air humidity − from 20 % to 80 %) with following measurement of nitrogen mass fraction.

Note: After opening the package the samples are selected for the measurement of mass fractions of nitrogen and moisture, the remaining portion of the sample material must not be stored.

Before carrying out the measurements, the package integrity is checking by means of visual observation. The package is opened and samples are selected.

8. Pilot laboratory

Laboratory of metrology of moisture measurement and certified reference material (241)

NMI's name and abbreviation

Ural Scientific Research Institute for Metrology, ROSSTANDART, Ekaterinburg (UNIIM)

The postal address: 4, Krasnoarmeiskaya St., Ekaterinburg, Russian Federation, 620000

Head of Laboratory 241, Maria Medvedevskikh
Telephone / Fax +7 (343) 350-60-63, 355-02-63
E-mail: Mariya.medvedevskikh@somet.ru

Research scientist lab. 241 Maria Krasheninina
Telephone / Fax +7 (343) 350-60-63, 355-02-63
E-mail: krasheninina_m@uniim.ru

9. References

- ISO 5537:2004 (IDF 26:2004) Dried milk –Determination of moisture content (Reference method).
- Cox M.G. The evaluation of key comparison data, Metrologia 39 (2002) 589-595
- Jorg W.Muller. Possible Advantages of a Robust Evaluation of Comparisons, Journal of Research of the National Institute of Standards and Technology Vol.105, No.4 (2000) 551-555
- Moore, J. C.; de Vries, W.; Lipp, M; Grifiths, J. C.; Abernethy, D. R. Total Protein Methods and Their Potential Utility to Reduce the Risk of Food Protein Adulteration. *Compr. Rev. Food Sci. Food Saf.* 2010. Vol. *9* (4), P. 330–351