Measurement uncertainty for the assigned value of the activity of Alkaline Phosphatase in lyophilized milk Reference Materials

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Introduction

The activity of phosphatase alkaline (ALP) is considered to be the main pasteurization tracer of milk. Reg. (CE) N° 1664/2006 establishes that ISO 11816-1 is the reference method and sets a limit for bovine ALP activity in pasteurized milk (<350 mU/L). Today, the own check system applied by food industry has become the key factor to demonstrate adherence to legislation on food safety. The reliability of the analytical results is crucial for the effectiveness of controls and Reference Materials (RM) are essential for analytical quality assurance. Nevertheless no commercial lyophilized RM for ALP in milk were, at the moment of the study, available.

The work describes the characterization and evaluation of RMs in milk produced by the National Reference Centre for bovine milk quality, to be used for the determination of ALP with the reference method ISO 11816-1: 2013. Samples were successfully validated in laboratory for their stability and homogeneity according to ISO 13528.

Homogeneity test

ANOVA	"60"		"600"		"6000"	
Significance value	0.018		0.00015		9.2E-09	
	mU/L	%	mU/L	%	mU/L	%
Sbb	2.19	2.3	14.77	1.9	173.37	2.9
Sr	2.37	2.5	9.94	1.3	64.40	1.1
Sr /Sbb influence	0.95	1.0	3.90	0.5	25.00	0.4

Stability test

Samples	Stability mean X	Homogeneity mean x	X-x	Target SD σ	0.3 σ
"60"	97.34	96.64	0.70	23.9	7.15
"600"	783.22	778.76	4.46	117.6	35.28
"6000"	5976.64	5973.70	2.94	1059.0	317.70

Scope

Production and values assignment of stabilized RMs at three levels of ALP activity corresponding to pasteurized, thermized and raw milk with standard uncertainties consistent with ISO 11816-1

ISO 11816-1

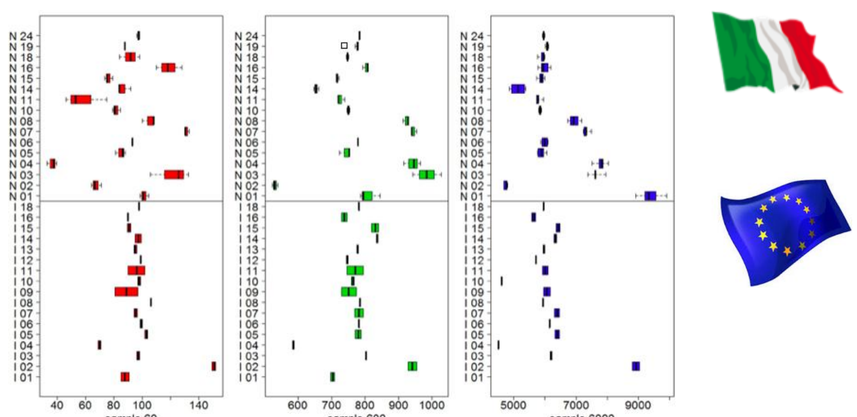
Values	Reproducibility limit ISO 11816-1: 2013	S_R %
< 125 mU/L	<23 mU/L in no more of 5% of cases	9.0 Calculated for sample "60"
125 mU/L< value< 620 mU/L	< 24 % in no more than of 5% of cases	8.6

Methodology

In summer 2014, the National Reference Laboratory (NRL) for Milk and Milk Products in collaboration with the National Reference Centre for bovine milk quality organized the first Italian national Proficiency Testing (PT) with these materials. The PT recruited 19 Italian laboratories (both public and private to reach the maximum possible number of participants). The EU Reference Laboratory for Milk and Milk Products joined it too. This step was the first attempt for the assignment of the titles and the uncertainties according to ISO 13528.

In 2015, as an improvement action, it was decided to organize a second round, using the same batch of samples used in 2014, thanks to their excellent stability. This new PT involved the EU Reference Laboratory for Milk and Milk Products and 17 well experienced NRLs from its network, with the scope to redefine reference values and relative uncertainties for the three levels of ALP activity. The assigned values (mU/L) of the first PT were confirmed and an important improvement of the uncertainties was reached.

Box-plot for 2014 and 2015 PTs



Comparisons of results from 2014 PT and 2015 PT

	2014 PT				2015 PT			
Samples	n	Assigned value (mU/L)	U%	ux (%)	n	Assigned value (mU/L)	U%	ux (%)
"60"	48	91	16.4	8.2	34	96	4.0	2.0
"600"	48	793	9.4	4.7	34	776	3.4	1.7
"6000"	96	6297	10.6	5.3	32	6041	4.2	2.1

Due to the fact that the same reference method was applied in both the rounds by all the participants, the experience of the laboratories resulted to be the main source of uncertainty.

The materials were fully characterized also according to ISO Guide 35 and proved to be highly stable over time (at moment they are tested for 3 years and tests are till in progress) and temperature, resulting an excellent candidate for internal instrumental quality control or for the organization of inter-laboratory trials for ALP in milk.

Characterization of RMs according to ISO Guide 35

Samples	2014 Italian trial				2015 European trial			
	Outliers/ n° observ.	Value (mU/L)	U (P95,K=2)	ux (%)	Outliers/ n° observ.	Value (mU/L)	U (P95,K=2)	ux (%)
"60"	12/48	90.9	9.0	4.9	8/34	96.7	5.2	2.7
"600"	9/48	759.9	65.1	4.3	4/34	775.0	34.3	2.2
"6000"	12/96	6267.1	567.7	4.5	6/32	6087.5	374.7	3.1

Conclusion

Based on the proved characteristics of stability, homogeneity and uncertainties, these RMs for milk produced through real exposure of ALP to heat treatment and not obtained by samples dilutions, represent a good internal quality control and, thanks to their excellent stability, an ideal tool for PTs in which samples shipment is a critical step.

The present work allowed to prove that the uncertainties of the materials were fit for the purpose.