

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

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Roles

National Reference Centre for bovine milk was designed by the national legislation D.M. 08.05.2002. It is mainly focused on qualitative improvement of milk, profilaxis of mastitis, payment system for milk quality and production and validation of RMs for quality controls in milk and milk products analyses

National Reference Laboratory for Milk and Milk Products was designated in 1997 by the national legislation DPR n.54 and reconfirmed by Reg (CE)882/2004. It operates at national level according to art 33: basically, collaboration with the EURL-MMP, coordination of the official laboratories activities and PT organization in dairy sector



Task

Activity of ALKaline Phosphatase (ALP) is considered to be the main **pasteurization tracer of milk**

Reg (CE) N° 1664/2006:

limit for ALP activity in **pasteurized bovine milk ≤ 350 mU/L**

Reference method

ISO 11816-1



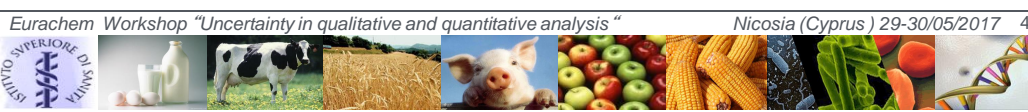
Criticalities

No stabilized Reference Materials are available for ALP, due to the measurand instability



PTs require:

- " Refrigerated transport of the samples
- " Fixed time for analysis



Need for RM

- “ Representative of real samples
- “ Different typologies of milk
- “ Different concentrations
- “ Homogeneous and stable
- “ Uncertainty fit for the purpose

Values	R ISO 11816-1	S _R %
<125 mU/L	< 23 mU/L in no more of 5% of cases	9.0 (at 91 mU/L)
125 mU/L < value < 620 mU/L	< 24% in no more of 5% of cases	8.6

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Samples preparation



Semi skimmed milk treatment	Expected concentration	Sample Cod
pasteurization 63 ° C - 30 ‘	< 350 mU/L	sample “60”
thermization 63 ° C - 15’	800 mU/L	sample “600”
dil 1:1000 with ALP free milk	< 7000 mU/L	sample “6000”

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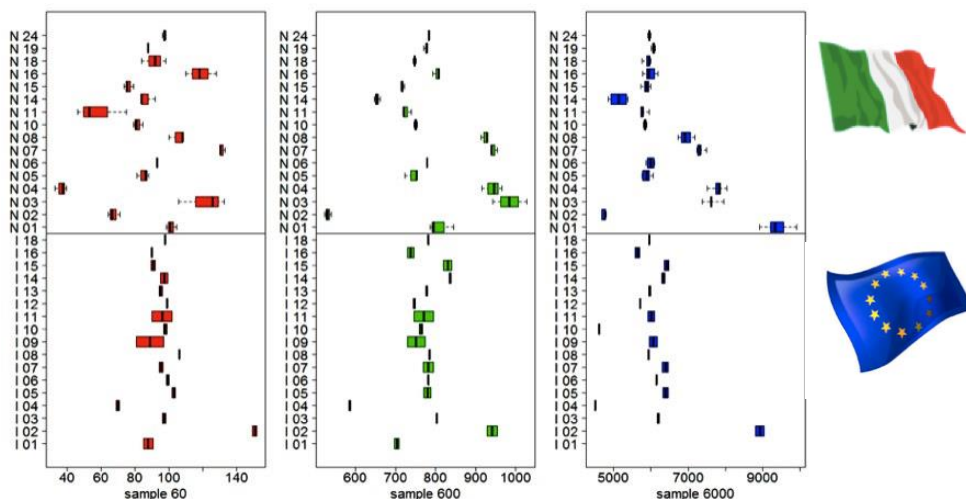


Characterization

2013	IZS-LER	Homogeneity and stability studies of the samples
2014	1° trial	Consensus value and robust SD from participants in a national PT round(18 labs and EURL-MMP) using the same method samples analyzed in triplicate
2015	2° trial	Consensus value and robust SD from participants in a european PT round (17 NRLs and EURL-MMP) using the same method samples analyzed in duplicate

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Results of inter laboratory trials



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Assigned values from PTs

Samples	2014 PT			2015 PT		
	n	Assigned value (mU/L)	u_x (%)	n	Assigned value (mU/L)	u_x (%)
60	48	91	8.2	34	96	2.0
600	48	793	4.7	34	776	1.7
6000	96	6297	5.3	32	6041	2.1

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Characterization of RMs according to ISO Guide 35

Sample	2014 Italian trial					2015 European trial				
	Outliers/ #Observations	value (mU/L)	U (p95, k=2)	U%	u_x %	Outliers/ #Observations	value (mU/L)	U (p95, k=2)	U%	u_x %
60	12/48	91	9	9.9	4.9	8/34	97	5	5.4	2.7
600	9/48	760	65	8.6	4.3	4/34	775	34	4.4	2.2
6000	12/96	6267	568	9.1	4.5	6/32	6088	375	6.2	3.1

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Conclusions

- RMs for milk were produced through **real exposure of ALP to heat treatment** and not by sample dilution
- **Homogeneity** was proven satisfactory
- For the first time, **excellent stability** was achieved (> 3 years) making shipment smart
- The uncertainties of the certified values were proven **fit for the purpose**



Thank you for your attention

