



Milk and milk products — Determination of alkaline phosphatase activity — Fluorimetric microplate method

TECHNICAL
SPECIFICATION

ISO/TS
4985

IDF/RM 255

First edition
2023-07



Determination of alkaline phosphatase activity: use, standard methods and challenges

IDF Webinar, Tuesday, 14 November 2023, Lotti Egger, Cédric Brügger

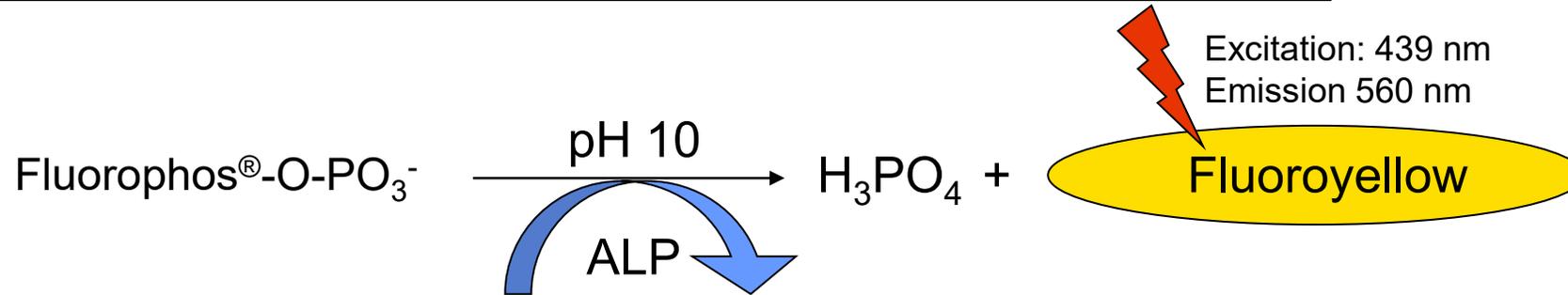


Introduction

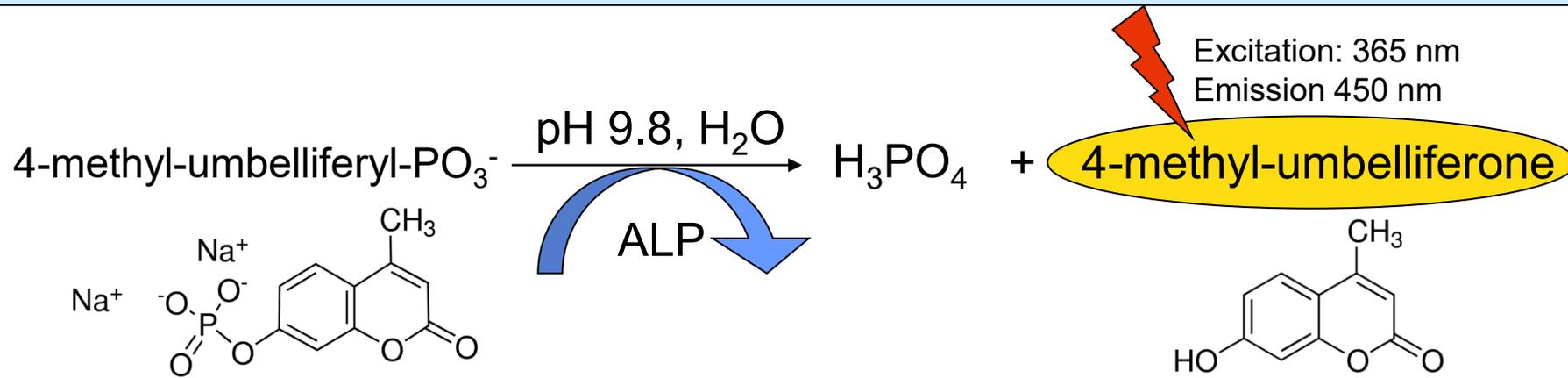
- Why a new ISO/IDF standard method for Alkaline Phosphatase activity?
 - To date, the available methods for the determination of ALP activity for official control are the reference method ISO 11816-1/IDF155-1 and ISO 22160/IDF 209. Both having the disadvantage of being tied to a single supplier in terms of material and reagents.
- What should this new method be capable of ?
 - Easy applicable methodology, with standard laboratory equipment and not entirely dependent on one supplier
 - Sensitive, with good repeatability and reproducibility
 - multiple samples to be run in parallel (possibility to automatize)
 - Good comparability with values of reference method ISO 11816
 - Low costs
 - ...

Method principles: Reference method_(ISO 11816) versus microplate ISO4985

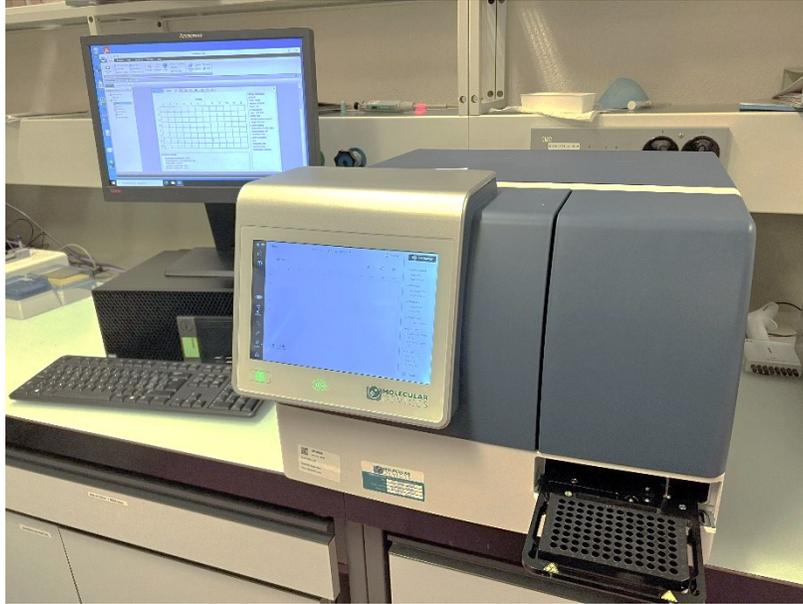
- Reference method: ISO11816-1 IDF155-1/ ISO11816-2 IDF155-2
- Instrument and Chemicals provided by Advanced Instruments



- Microplate method: ISO4985/ IDF 255 → **Open method (non-proprietary)**



ISO/IDF 4985/255 Microplate ALP method: Equipment and assay protocol



- **Instrument:**

- Fluorescence microplate reader (e.g. Molecular Device, Biotek, Labtech, Millipore...) equipped with fluorescence filters excitation/emmission at 365/450 nm
- Black 96 well plates

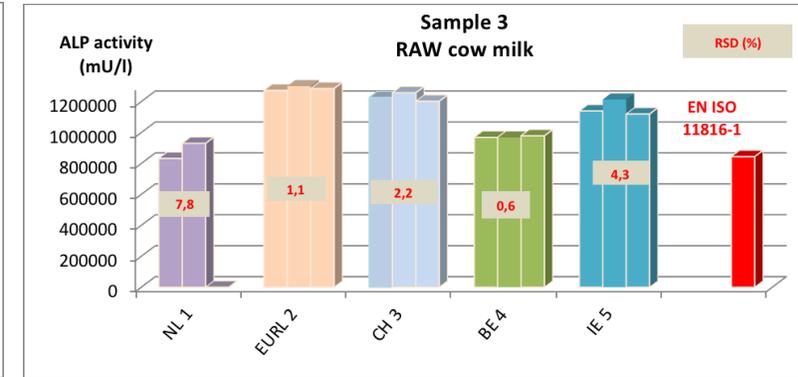
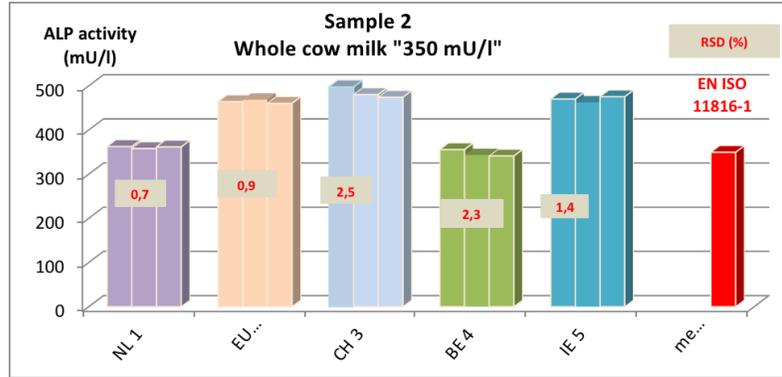
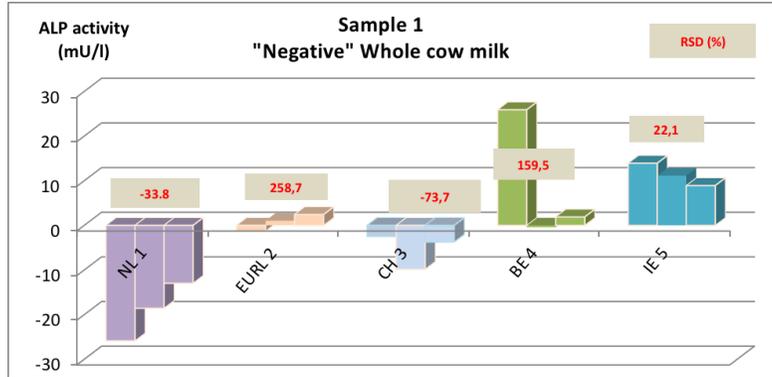
- **Kinetic Assay in microplate:**

- Sample preparation: → milk: no preparation; → cheese: similar as in the reference method ISO 11816-2
- Primary sample dilution 1:10 in 2-amino-2-methyl-1-propanol (AMP) buffer
- Generation of blank by heating one portion of a sample of the same matrix at 95°C for 5 min
- Pipetting 100 μ L per well of sample and blank in triplicates
- Addition of 100 μ L of substrate 4-MU-Phosphate
- Kinetic fluorescence measurement (365/450 nm) at 37°C for 15 min
- Calculation of slope from linear part of the curve
- Calculation of activity by using the standard curve measured with the fluorescent product 4-MU

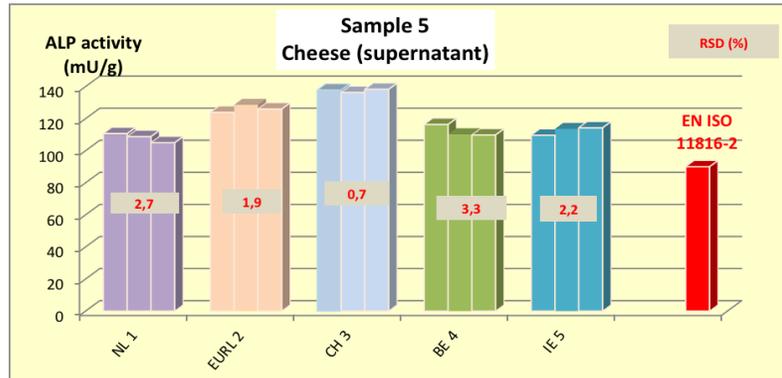
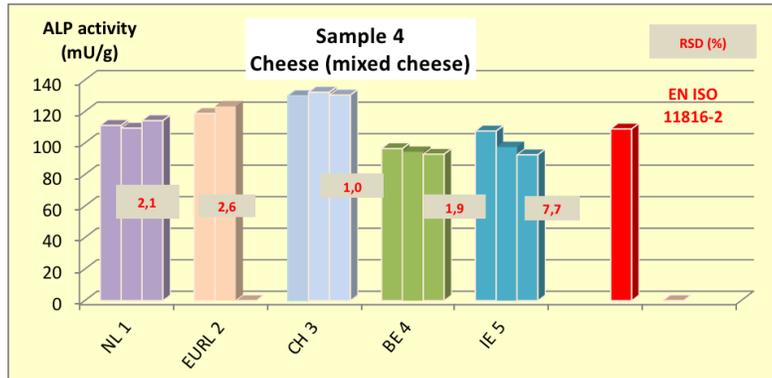


Pilot study: April 2017

milk



cheese



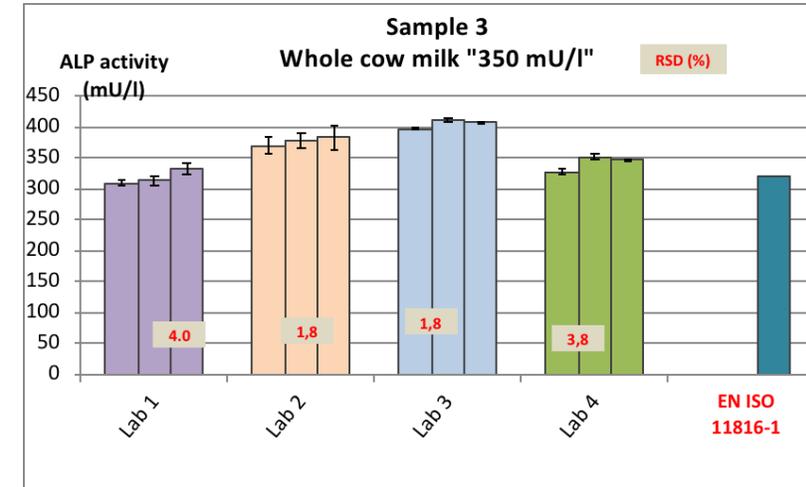
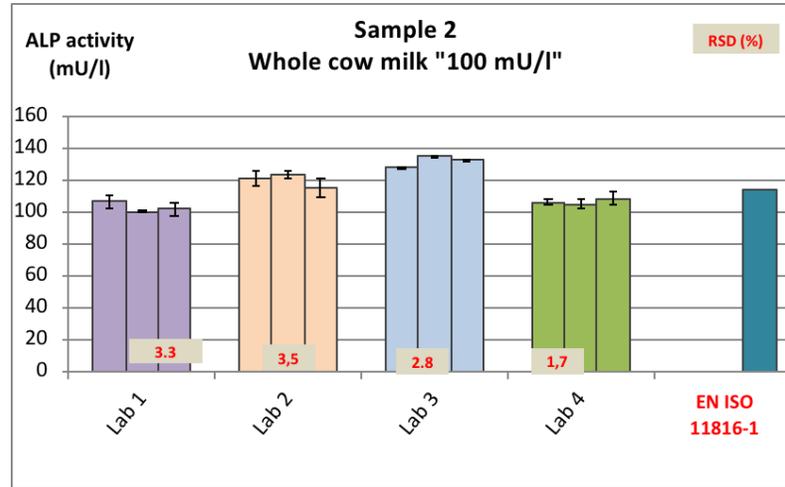
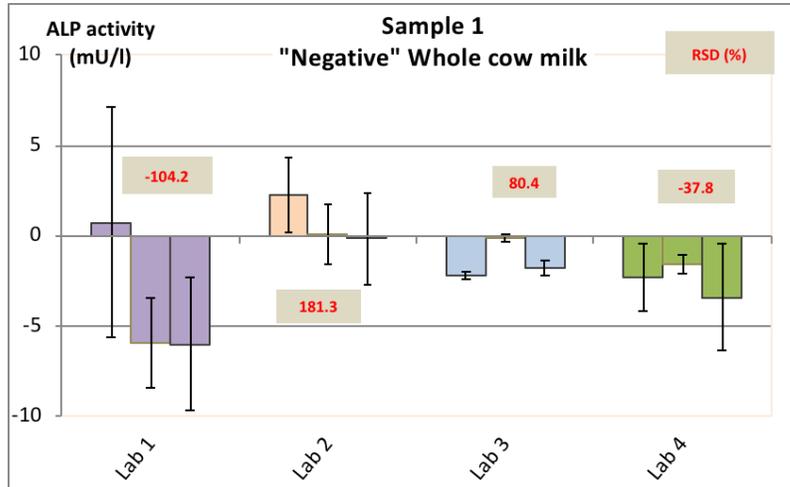
Samples:
3 different milk samples
2 cheeses

Participation:
5 labs, 5 countries

→ Good reapeatability and good comparability with ISO 11816-1 and 11816-2 for milk and cheese samples



Collaborative study: September 2017



Samples: 3 different milk samples; Participation: 4 labs, 4 countries

- Again: good reapeatability and good comparability with ISO 11816-1 and 11816-2
- But: not enough participants for the validation of a full ISO/IDF standard method
- Publication as TS ISO/IDF 4985/255 in 2023



Statistical evaluation of ISO/IDF 4985/255

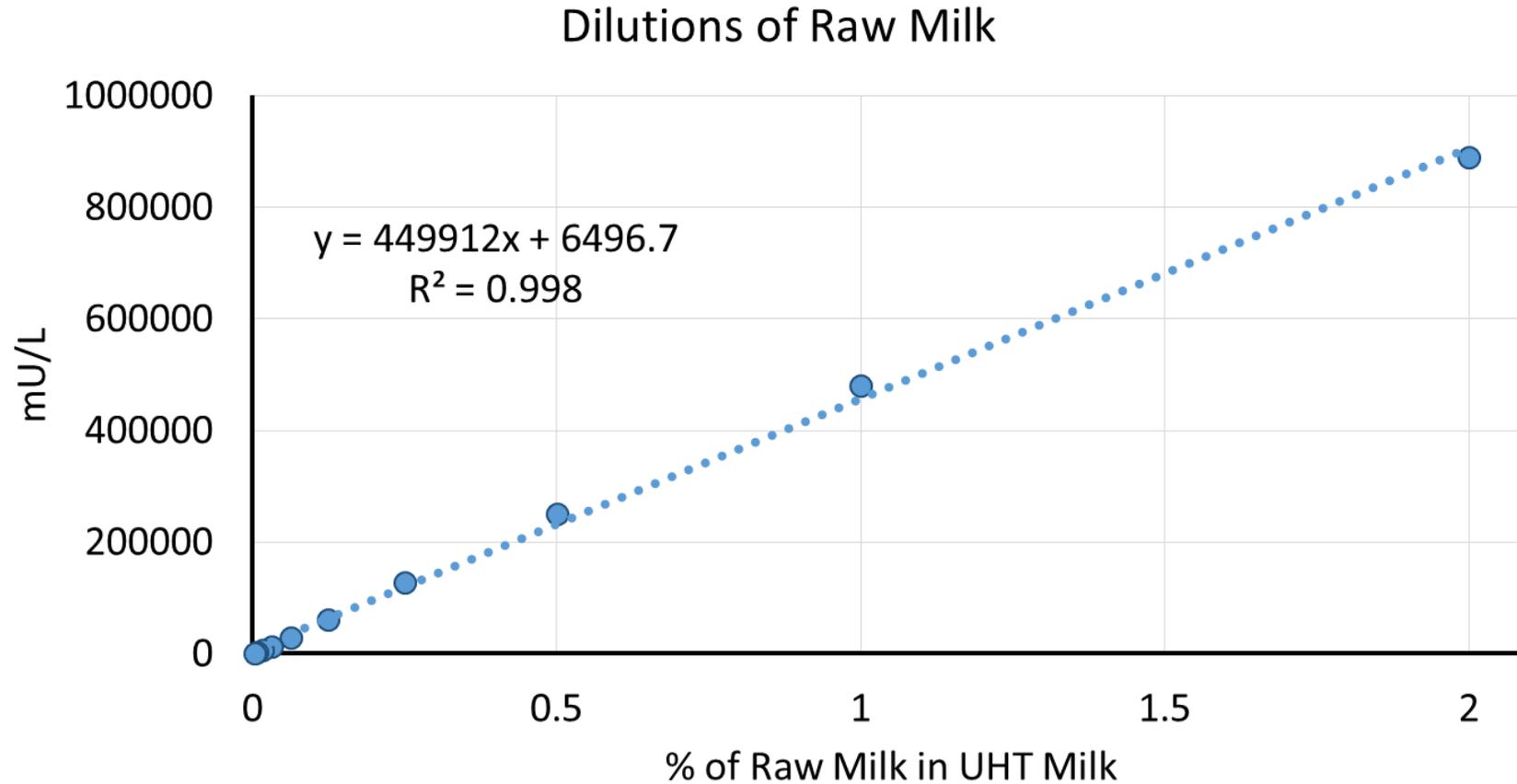
Microplate method (ISO TS 4985-2023)							
lab code	Milk (mU/l)		Cheese (mU/g)		lab code	Milk (mU/l)	
	sample 2	sample 3	sample 4	sample 5		sample 2	sample 3
April					Sept.		
1	360.81	4405.99	111.66	108.14	3	131.5	404.8
2	464.47	6428.62	121.04	126.17	4	106.0	342.7
3	483.99	6141.69	130.91	137.44	2	119.6	376.8
4	346.78	4858.82	94.63	112.14			
5	469.41	5787.86	99.26	112.50	1	102.7	318.7
p =	5	5	5	5		4	4
average	425.09	5524.59	111.50	120.78		114.92	360.76
std dev	65.67	860.51	15.02	12.23		13.23	37.79
sr =	7.36	179.83	4.07	2.63		3.38	10.35
relative sr =	1.73%	3.21%	3.67%	2.21%		2.94%	0.03
r =	20.61	503.52	11.40	7.38		9.46	28.98
relative repeatability=	4.8%	9.0%	10.3%	6.2%		8.2%	8.0%
sR =	65.94	835.38	15.65	12.42		13.51	38.72
relative sR =	15.51%	14.91%	14.12%	10.41%		11.76%	10.73%
R =	184.64	2339.05	43.81	34.77		37.84	108.43
relative reproducibility=	43.4%	41.7%	39.5%	29.2%		32.9%	30.1%
Pilot study				Collabo- rative study			

Fluorophos (Milk: ISO 11816-1:2022; Cheese: ISO 118-2:2022)								
Milk target value mU/L				Cheese (mean value mU/g)				
40	100	350	500	994	1974	136	4408	2608
11	17	32	37	115	194	14	732	373
10%	5%	3%	2%	12%	10%	10%	17%	14%

→ Similar repeatability and reproducibility for milk and cheese samples



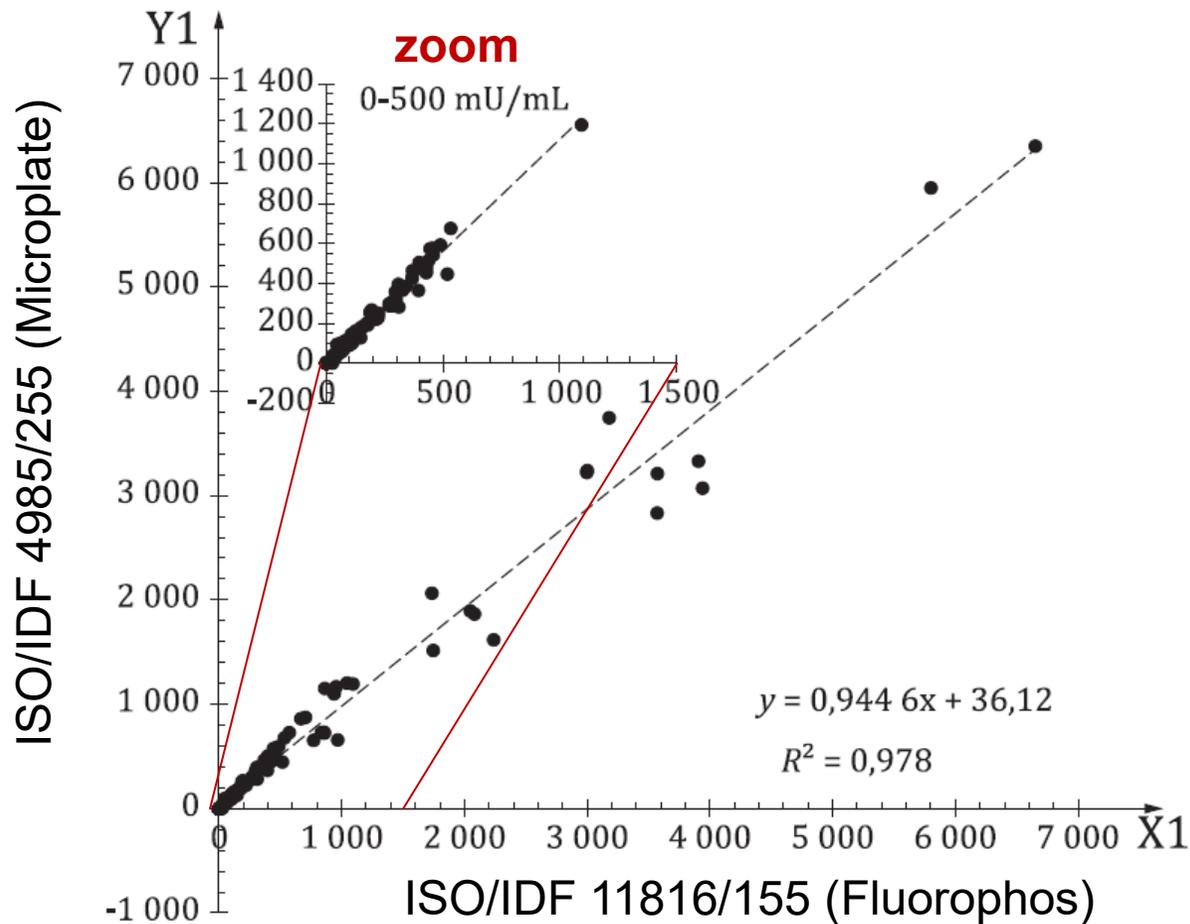
Sensitivity: Lower limit of detection



Detection of 0.003 % of Raw Milk in UHT Milk possible → similar as Reference method
Calculated limit of detection: 15 mU/L



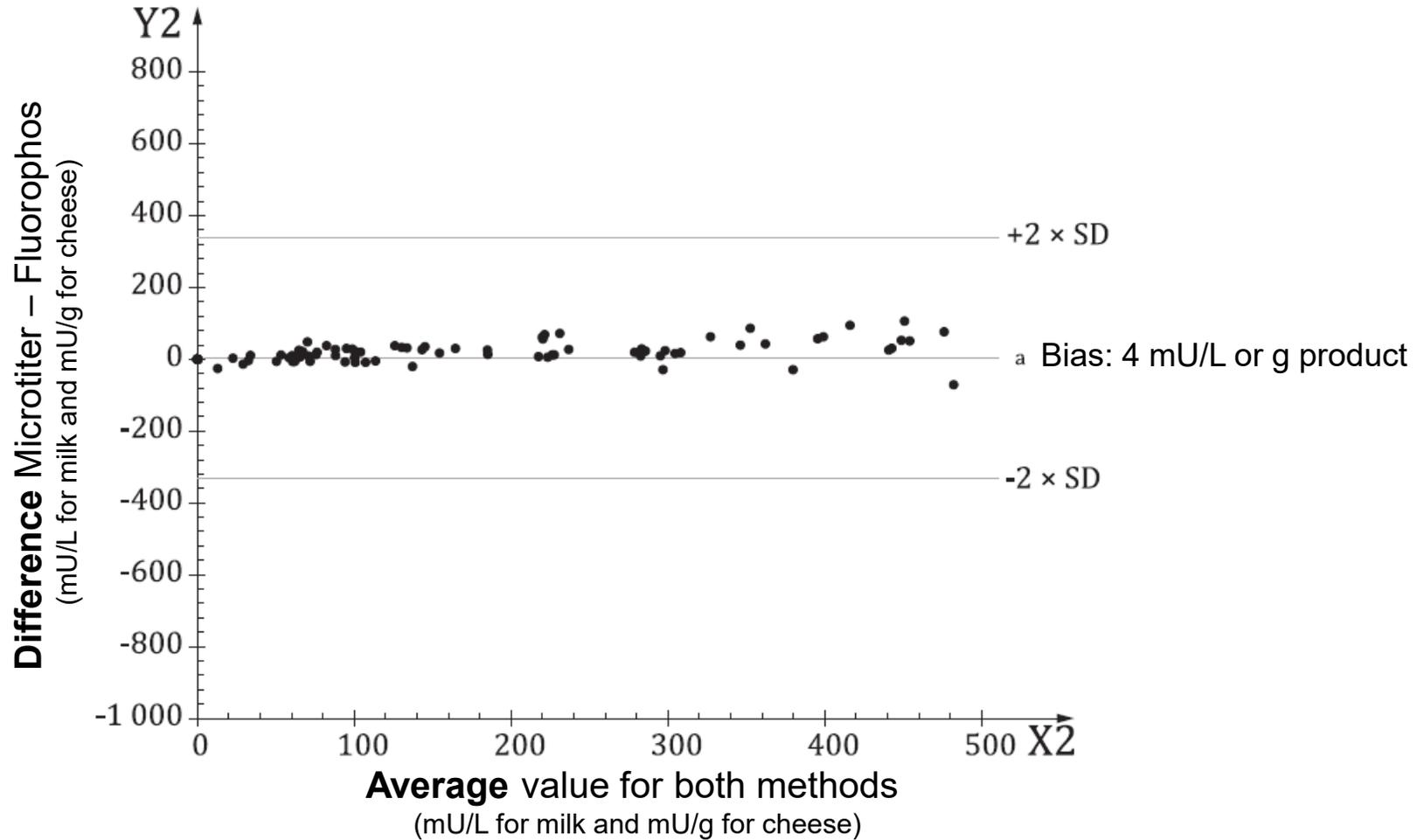
Method Comparison I: ISO/IDF 4985/255 versus Reference method



High correlation between ISO/IDF 4985/255 and the reference method ISO/IDF 11816/155: R^2 0.978, slope 0.944, including milk and cheese samples



Method Comparison II: Reference method versus ISO/IDF 4985/255

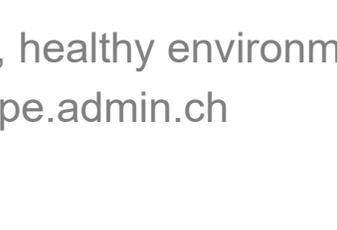


Average expected bias between methods: 4 mU/L or mU/g of product



Summary

- ISO/IDF TS 4985/255 Microplate ALP method is an open method with commercially available products and equipment, allowing multiple samples to be run in parallel
 - Repeatability and reproducibility in milk and cheese was demonstrated in a pilot study with 5 labs and in a collaborative study with 4 labs
 - ALP activities obtained with ISO /IDF 4985/255 (microplate) and ISO/IDF 11816/155 (Fluorophos) are highly correlated: R^2 0.98, slope 0.94) and are within 2 Standard deviations in >95 % of cases applying the Bland-Altman method for comparison
 - ISO/IDF 4985/255 is at the moment available as TS
- As soon as enough laboratories are willing to participate, a collaborative study will be organized in order to publish the method as full ISO/IDF standard



Thank you for your attention

Lotti Egger

charlotte.egger@agroscope.admin.ch

Agroscope good food, healthy environment

www.agroscope.admin.ch

