Maisons-Alfort Laboratory
For Food Safety

European Union Reference Laboratory for Milk and Milk Products

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DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY IN COW MILK

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DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY IN COW MILK

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CONTENT

1. INTRODUCTION

The Laboratory for Food Safety, Maisons-Alfort site, of Anses (French agency for food, environmental and occupational health safety), European Union Reference Laboratory (EURL) for Milk and Milk Products, organised an inter-laboratory trial for the National Reference Laboratories (NRLs) to assess their ability to determine the alkaline phosphatase activity (ALP) in cow milk.

The method to apply was the method prescribed for official controls: i.e. the European and International Standard EN ISO 11816-1: 2006 “Milk and milk products – Determination of alkaline phosphatase activity – Part 1: Fluorimetric method for milk and milk-based drinks”.

2. GENERAL INFORMATION

2.1 PARTICIPANTS

Twenty one National Reference Laboratories (NRLs) participated to this proficiency trial.
Laboratories were anonymously and individually numbered.
Participating laboratories are given in ***Appendix I.***

2.2 ORGANISATION OF THE PROFICIENCY TESTING TRIAL - INSTRUCTIONS TO PARTICIPANTS

Laboratories were informed of the organization of a Proficiency Test (PT), by a circular letter sent out in October 2012. The circular letter gave information about the method to apply, the number of samples to analyze and the date of analysis.

The instructions to participants’ sheet, the acknowledgement of receipt and the results form were forwarded by email to all participants in advance of the trial, and then again with the samples shipped by express courier.

In the instructions sheet, participants' attention was drawn to the need to strictly stick to the reference method and laboratories were clearly instructed to perform one single analysis per sample. Participants were asked to analyze the samples on 30 November 2012 (one determination per sample) and to report results in mU of Alkaline Phosphatase activity per liter of milk (to one decimal place).

NRLs were requested to submit results by 12 December 2012, at the latest.

The samples were dispatched on 27 November 2012.

Each participating laboratory received, by express courier, in a thermo box:
- 3 series of 6 individual samples
- 3 “negative” samples (to calibrate the instrument)
- 1 “shipment sample” (to check the temperature upon receipt)

The samples were labelled as follows:

Samples	Sample matrix	Labelling
1st series	Whole Cow milk	Sample n° ***Whole***
2nd series	Semi-skimmed Cow milk	Sample n° ***Semi-skimmed***
3rd series	Skimmed Cow milk	Sample n° ***Skimmed***
« Negative » samples	Whole Cow milk	*Whole Cow milk* Negative Sample for calibration
	Semi-skimmed Cow milk	*Semi-skimmed Cow milk* Negative Sample for calibration
	Skimmed Cow milk	*Skimmed Cow milk* Negative Sample for calibration
Shipment sample	Water	Shipment sample H_2O

19 laboratories (90% of the participants) received the parcel the day after the dispatch and 1 within two days. Laboratory 11 never sent the acknowledgment of receipt (AR) though we know through the express courier web page that the parcel arrived in due time.
Only 8 laboratories sent their AR upon receipt.

All laboratories received the samples in good condition and temperatures recorded were satisfactory since they ranged between 2,5°C and 12°C, and the temperature limit was set to 15°C.
Deadline for reporting results was respected by all the participants but the results sheet was unfortunately not always duly filled (signatures were missing, incorrect transcription of the method reference).

3. PROFICIENCY TEST ITEMS

3.1 PREPARATION OF THE PROFICIENCY TEST ITEMS

Three types of cow milk (whole, semi-skimmed and skimmed) were prepared by EURL. Each type consisted of two different levels of AP, level “1” and “2”, plus a “negative” sample: level “0”. Samples were prepared in blind duplicates and shipped under cold conditions.

Test Samples	ALP Levels	Number of samples
Whole Cow Milk	“0”, “1” and “2”	6 *individual samples* = 3 samples x 2 (corresponding to blind duplicates)
Semi-skimmed Cow Milk	“0”, “1” and “2”	6 *individual samples* = 3 samples x 2 (corresponding to blind duplicates)
Skimmed Cow Milk	“0”, “1” and “2”	6 *individual samples* = 3 samples x 2 (corresponding to blind duplicates)

The negative samples, level "0" (i.e. ALP-free milk samples) were commercial cow milk (whole and semi-skim pasteurized milk and skimmed UHT milk) heated at 95°C for 5 minutes so as to obtain an ALP activity < 10 mU/l (according to standard EN ISO 11816-1: 2006 [1]).
The other samples were prepared by addition of micro-filtered milk in the previously mentioned negative samples aiming to obtain activity levels of approximately 190 and 250 mU/l.

For whole milk samples, micro-filtered whole milk was added to negative pasteurized whole milk; for semi-skim milk and skim milk samples, micro-filtered semi-skim milk was added to negative pasteurized semi-skim milk and to negative UHT skim milk, respectively.

Each level batch was thoroughly mixed with a magnetic stirrer before being distributed in stoppered glass vials of 4 ml.

The "negative samples for calibration" were ALP-free milk samples packed in glass vials of 15 ml.

Glass vials were individually labelled by randomization and stored at 5±3°C prior to shipment.

3.2 IDENTIFICATION OF THE PROFICIENCY TEST ITEMS

The codification of the samples is an encrypted random coding including all samples with different levels (samples with different levels to be sent to participants, samples for the homogeneity test and additional samples).

The samples' coding is presented in ***Appendix II.***

3.3 HOMOGENEITY

Homogeneity was tested according to the statistical procedure proposed in the 2006 IUPAC Harmonized protocol for PT[2]. Ten vials selected strictly at random were analyzed in duplicate under repeatability conditions.

In this procedure, the between sample variability is determined and compared to a combination of fitness for purpose and statistical criteria.

"Sufficient homogeneity" requires that,

$$s^2_{sam} \leq F_1 (0{,}3 \times \hat{\sigma}) + F_2 s^2_{an}$$

where $\hat{\sigma}$ *: target standard deviation,*

s^2_{sam} *and* s^2_{an} *: estimates of sampling and analytical variances obtained from an ANOVA,*

F_1 *and* F_2 *: constants derived from standard statistical tables.*

[1] EN ISO 11816-1 : 2006 "Milk and milk products – Determination of alkaline phosphatase activity – Part 1: Fluorimetric method for milk and milk-based drinks"

[2] Thompson M, Ellison SLR, Wood R. "The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" (IUPAC Technical Report) *Pure Appl Chem* (78) 1:145-196, 2006

The target standard deviation is determined using the repeatability and reproducibility standard deviations (S_r *and* S_R) from a collaborative study described in the Draft International Standard ISO/DIS 11816-1 and is calculated as follows:

$$\hat{\sigma} = \sqrt{S_R^2 + S_r^2 \times \frac{1 - n}{n}}$$

S_r and S_R correspond, respectively, to 12% and 24% of the mean of the results obtained during the homogeneity test.

For the negative samples, no statistical test is applied, the ALP contents of all determinations should be less than 10 mU/l. On this basis, the negative samples were also declared homogeneous.

3.4 STABILITY

A preliminary study on samples' stability had been implemented. The tests were done according to ISO 13528: 2005[3]. Three vials selected strictly at random were analyzed in duplicate under repeatability conditions. "Sufficiently stable" samples implies that the following formula should be satisfied

$$|\bar{x} - \bar{y}| \leq 0{,}3\ \hat{\sigma}$$

Where $\bar{x}$ *= parametric mean of the measurements obtained during the homogeneity test,*
$\bar{y}$ *= parametric mean of the measurements obtained during the stability test,*
$\hat{\sigma}$ *= target standard deviation (same as for homogeneity test)*

The preliminary study demonstrated limited stability; consequently we decided to fix a mandatory date for the analyses.

4. RESULTS

4.1 ASSESSMENT CRITERIA

4.1.1 Non statistical criteria

4.1.1.1 Respect of the instructions

Laboratories not respecting the instructions received, i.e. respect of the prescribed date of analysis and respect of the method's specifications (particularly System and Reagent Controls) were excluded from the evaluation, as cited in the Instructions to participants' form, under "Criteria for refusing results".

[3] ISO 13528:2005 : "Statistical methods for use in proficiency testing by interlaboratory comparisons"

4.1.1.2 Respect of the repeatability.

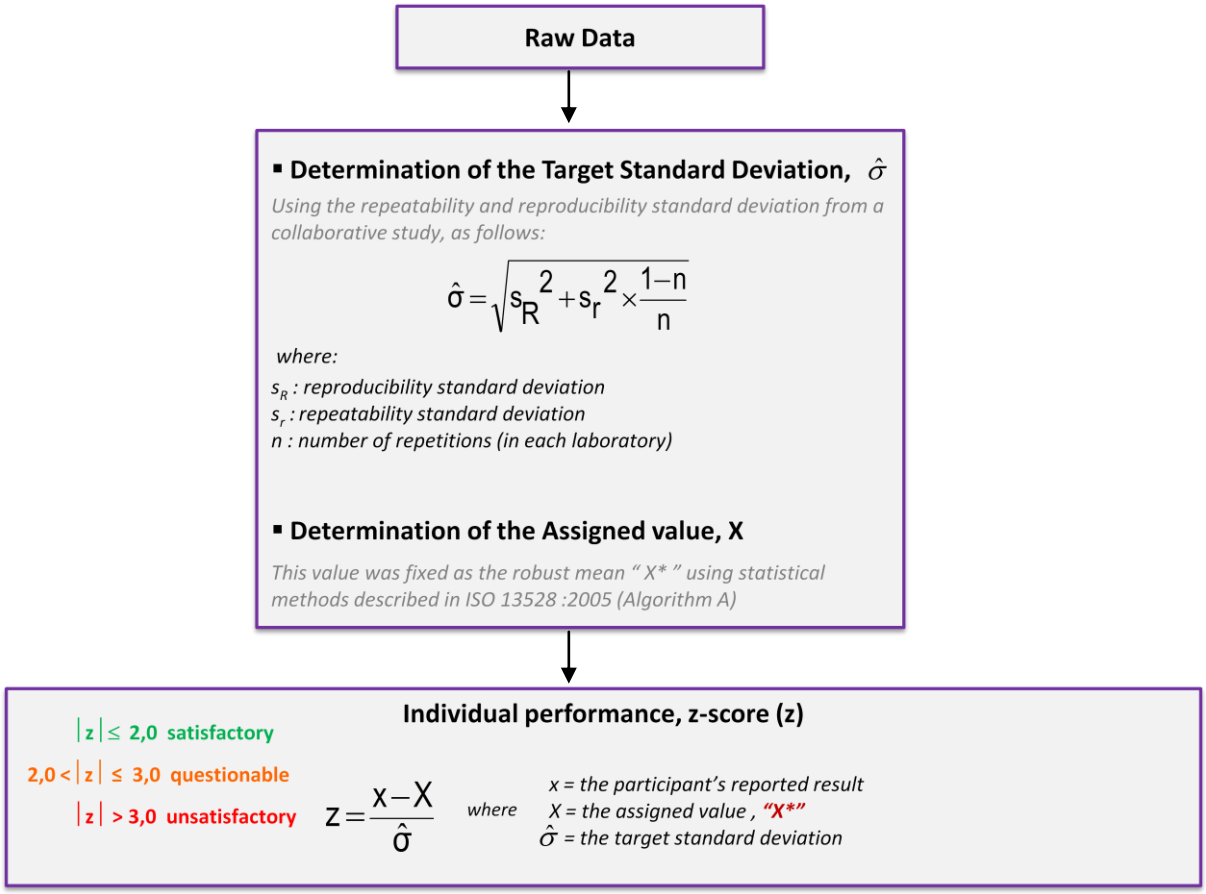
Considering our particular scope, i.e. PT for a specific method, respect of repeatability is deemed to be an essential requirement. Thus, non respect of the repeatability value was evaluated as an unsatisfactory performance, as mentioned in the Instructions to participants' form, under "Decision rules for accepting results".

The eighteen individual samples sent to participants were, in fact, blind duplicates of 9 samples.
Consequently, the difference obtained between the blind duplicates was assessed against the repeatability value.
Repeatability limit (r) corresponds to 12% of the mean of the two determinations obtained by the participating laboratory (ISO/DIS 11816-1).

4.1.2 Statistical criterion for the evaluation of individual performance of the laboratories

4.1.2.1 Overview

The evaluation of the results was carried out in several stages summarized below:



4.1.2.2 Target standard deviation, $\hat{\sigma}$

$\hat{\sigma}$ is used to determine the limits of satisfactory performance in proficiency testing.

The target standard deviation for the performance evaluation was calculated as follows (Standard ISO 13528: 2005[4]):

$$\hat{\sigma} = \sqrt{S_R^2 + S_r^2 \times \frac{1-n}{n}}$$

Where s_R*: reproducibility standard deviation*
s_r*: repeatability standard deviation*
n: number of repetitions (in each laboratory)

S_r and S_R are derived from the limits of repeatability and reproducibility, corresponding to 12% and 24% of the robust mean, X*, obtained by the NRLs network.

4.1.2.3 Assigned value, X

This value is used for the performance evaluation of the laboratories.
In this study, according to ISO 13528: 2005[4], "X" was fixed as the robust mean "X*" of all results submitted.

4.1.2.4 Trueness - Individual *z*-scores

The evaluation criterion for the individual performance is the trueness of each participant measured by the systematic deviation between its results and the assigned value for each sample of the trial.

To assess this variation, the proficiency testing results are usually transformed into performance statistics which facilitate the interpretation and the comparison of the results.

We have chosen to convert the results obtained by each participant into "*z*-score".

The *z*-score is calculated as follows:

$$z = \frac{x - X}{\hat{\sigma}}$$

Where x *= the participant's reported result, individual result (one replicate)*
X *= the assigned value*
$\hat{\sigma}$ *= the target standard deviation*

The z-scores interpretation was performed according to Standard ISO 13528: 2005[4]. The performance of each laboratory was estimated from the absolute value of |z| and was evaluated as follows:

$\lvert z \rvert \leq 2{,}0$	satisfactory
$2{,}0 < \lvert z \rvert \leq 3{,}0$	questionable
$\lvert z \rvert > 3{,}0$	unsatisfactory

[4] ISO 13528:2005 : "Statistical methods for use in proficiency testing by interlaboratory comparisons"

4.2 RESULTS & EVALUATION

4.2.1 Raw data: compilation of all data received

Level	Whole - 0		Whole - 1		Whole - 2		Semi skim - 0		Semi skim - 1		Semi skim - 2		Skim - 0		Skim - 1		Skim - 2	
Lab. code	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2
1																		
2	<10	<10	218,6	170,5	256,6	249,2	<10	<10	188,9	176,6	285,2	245,1	<10	<10	163,0	162,1	249,0	226,6
3	<10	<10	203,2	205,0	271,2	271,2	<10	<10	209,6	217,9	290,5	282,3	<10	<10	200,0	203,6	263,4	265,2
4	<10	<10	181,6	179,7	237,2	237,2	<10	<10	190,8	186,6	246,4	250,5	<10	<10	167,8	170,1	228,5	230,3
5	<10	<10	311,7	303,9	440,4	409,6	<10	<10	331,0	313,1	428,9	446,8	<10	<10	305,2	293,7	400,4	416,0
6	17,0	12,4	167,3	167,3	235,8	232,6	10,1	11,5	184,8	188,0	256,1	251,0	<10	<10	174,2	176,1	237,7	233,1
7	15,6	12,0	191,7	182,0	254,2	263,9	<10	16,1	195,4	185,7	251,9	250,1	<10	11,5	177,0	175,1	229,4	237,2
8	<10	<10	205,9	187,6	253,8	255,6	<10	<10	192,6	190,8	258,4	250,1	<10	<10	160,9	157,2	211,9	227,1
9	<10	<10	216,5	204,6	273,5	277,7	<10	<10	208,2	212,4	281,8	277,7	<10	<10	194,9	189,4	264,8	268,5
10	11,0	11,0	193,1	204,6	249,2	265,7	12,0	17,9	217,4	213,3	269,8	277,7	<10	11,0	176,5	174,7	241,3	228,9
11																		
12	19,3	15,6	203,2	195,4	265,7	263,4	12,0	12,0	200,0	196,0	263,4	269,4	<10	<10	197,2	182,5	252,4	248,7
13	24,4	17,5	217,9	211,0	290,5	283,6	12,9	12,9	221,6	223,9	304,3	276,3	<10	<10	212,4	218,8	288,2	285,9
14	<10	<10	194,0	195,8	256,1	256,1	<10	<10	194,5	194,5	262,0	253,3	<10	<10	201,8	201,8	255,6	264,3
15																		
16	12,4	16,1	194,9	193,1	255,1	260,6	11,5	11,5	202,3	194,5	283,2	274,0	<10	<10	170,1	168,3	236,7	244,1
17	15,2	11,5	191,7	189,9	248,2	244,6	15,2	11,5	191,7	191,7	258,4	256,5	<10	<10	189,9	189,9	245,9	245,9
18	<10	<10	182,2	184,3	246,9	248,9	<10	<10	190,8	192,6	252,9	254,7	<10	<10	173,8	169,6	239,0	237,2
19	20,7	<10	199,1	191,7	265,7	265,7	<10	<10	200,9	187,1	257,9	260,2	<10	<10	178,8	184,8	260,2	250,5
20	<10	<10	176,5	176,5	242,3	234,4	<10	<10	183,4	181,1	240,0	247,3	<10	<10	167,3	171,0	224,3	231,2
21	<10	<10	223,0	208,2	230,3	273,1	<10	<10	228,0	214,7	254,7	279,0	<10	<10	211,5	223,0	265,7	269,4

Table 1- Raw data (mU/l)

Failed to submit results

Deadline of analysis not respected

Prescription for system and reagent controls not respected

Non respect of the method prescribed

For the ALP target level "0" (negative samples), the instrument readings should be "<10" indicating that no fluorescence activity was detected.

But according to our experience, heated cow's milk may sometimes show activities up to 20 mU/l; such samples can still be considered as negative.

Results submitted by participants for this level were satisfactory.

4.2.2 Non-statistical evaluation

4.2.2.1 Respect of the method instructions

Below are presented the results of the verification of instrument performance, system and reagent controls.

Laboratory Code	System and Reagent Controls			PhosphaCheck Controls		
	302	**602**	**Substrate**	**Negative**	**Normal**	**Positive**
	(302±4 FLU)	*(602±12 FLU)*	*(<1200 FLU)*	*(<10 mU/l)*	*(<40 mU/l)*	*(500±100 mU/l)*
2	298	598	919	<10	29	517,2
3	305	604	726	<10	34,9	504,8
4	306	602	**1352**	<10	31,7	559,9
5	**309**	602	775	<10	**55,2**	**828,8**
6	306	595	829	<10	20,2	427,5
8	302	602	653	<10	15,6	470,7
9	306	601	969	<10	26,7	560,4
10	298	600	880	<10	20,2	520,4
12	303	606	692	<10	17,9	571,9
13	306	598	561	<10	25,3	578,8
14	306	591	650	<10	24,4	527,3
16	300	598	700	<10	17	553,5
17	306	605	625	<10	22,5	508,9
18	302	604	735	<10	26,7	540,1
19	304	594	681	<10	21	555
20	301	609	783	<10	24,4	497,4
21	302	605	820	<10	24	553

Table 2- Results of control tests

Prescription for system and reagent controls not respected

Laboratory 4 was excluded from the evaluation because of non-respect of the range prescribed for the substrate.
Laboratory 5 was excluded from the evaluation because the value obtained for the daily A/D test was outside the compliance range.

4.2.2.2 Respect of the repeatability value

Level	Level 1					Level 2				
Lab. code	rep.1	rep.2	*diff.*	mean	r	rep.1	rep.2	*diff.*	mean	r
2	218,6	170,5	***48,1***	194,6	**23,3**	256,6	249,2	*7,4*	252,9	30,3
3	203,2	205,0	*1,8*	204,1	24,5	271,2	271,2	*0,0*	271,2	32,5
6	167,3	167,3	*0,0*	167,3	20,1	235,8	232,6	*3,2*	234,2	28,1
8	205,9	187,6	*18,3*	196,8	23,6	253,8	255,6	*1,8*	254,7	30,6
9	216,5	204,6	*11,9*	210,6	25,3	273,5	277,7	*4,2*	275,6	33,1
10	193,1	204,6	*11,5*	198,9	23,9	249,2	265,7	*16,5*	257,5	30,9
12	203,2	195,4	*7,8*	199,3	23,9	265,7	263,4	*2,3*	264,6	31,7
13	217,9	211,0	*6,9*	214,5	25,7	290,5	283,6	*6,9*	287,1	34,4
14	194,0	195,8	*1,8*	194,9	23,4	256,1	256,1	*0,0*	256,1	30,7
16	194,9	193,1	*1,8*	194,0	23,3	255,1	260,6	*5,5*	257,9	30,9
17	191,7	189,9	*1,8*	190,8	22,9	248,2	244,6	*3,6*	246,4	29,6
18	182,2	184,3	*2,1*	183,3	22,0	246,9	248,9	*2,0*	247,9	29,7
19	199,1	191,7	*7,4*	195,4	23,4	265,7	265,7	*0,0*	265,7	31,9
20	176,5	176,5	*0,0*	176,5	21,2	242,3	234,4	*7,9*	238,4	28,6
21	223,0	208,2	*14,8*	215,6	25,9	230,3	273,1	***42,8***	251,7	**30,2**

Table 3- Data (mU/l) for Whole Cow Milk samples:
Raw data, Difference between duplicates, mean and "individual" repeatability limit

Repeatability non respected

Level	Level 1					Level 2				
Lab. code	rep.1	rep.2	*diff.*	mean	r	rep.1	rep.2	*diff.*	mean	r
2	188,9	176,6	*12,3*	182,8	21,9	285,2	245,1	***40,1***	265,2	**31,8**
3	209,6	217,9	*8,3*	213,8	25,7	290,5	282,3	*8,2*	286,4	34,4
6	184,8	188,0	*3,2*	186,4	22,4	256,1	251,0	*5,1*	253,6	30,4
8	192,6	190,8	*1,8*	191,7	23,0	258,4	250,1	*8,3*	254,3	30,5
9	208,2	212,4	*4,2*	210,3	25,2	281,8	277,7	*4,1*	279,8	33,6
10	217,4	213,3	*4,1*	215,4	25,8	269,8	277,7	*7,9*	273,8	32,9
12	200,0	196,0	*4,0*	198,0	23,8	263,4	269,4	*6,0*	266,4	32,0
13	221,6	223,9	*2,3*	222,8	26,7	304,3	276,3	*28,0*	290,3	34,8
14	194,5	194,5	*0,0*	194,5	23,3	262,0	253,3	*8,7*	257,7	30,9
16	202,3	194,5	*7,8*	198,4	23,8	283,2	274,0	*9,2*	278,6	33,4
17	191,7	191,7	*0,0*	191,7	23,0	258,4	256,5	*1,9*	257,5	30,9
18	190,8	192,6	*1,8*	191,7	23,0	252,9	254,7	*1,8*	253,8	30,5
19	200,9	187,1	*13,8*	194,0	23,3	257,9	260,2	*2,3*	259,1	31,1
20	183,4	181,1	*2,3*	182,3	21,9	240,0	247,3	*7,3*	243,7	29,2
21	228,0	214,7	*13,3*	221,4	26,6	254,7	279,0	*24,3*	266,9	32,0

Table 4- Data (mU/l) for Semi-Skimmed Cow Milk samples
Raw data, Difference between duplicates, mean and "individual" repeatability limit

Repeatability non respected

Level	Level 1					Level 2				
Lab. code	rep.1	rep.2	*diff.*	mean	r	rep.1	rep.2	*diff.*	mean	r
2	163,0	162,1	*0,9*	162,6	19,5	249,0	226,6	*22,4*	237,8	28,5
3	200,0	203,6	*3,6*	201,8	24,2	263,4	265,2	*1,8*	264,3	31,7
6	174,2	176,1	*1,9*	175,2	21,0	237,7	233,1	*4,6*	235,4	28,2
8	160,9	157,2	*3,7*	159,1	19,1	211,9	227,1	*15,2*	219,5	26,3
9	194,9	189,4	*5,5*	192,2	23,1	264,8	268,5	*3,7*	266,7	32,0
10	176,5	174,7	*1,8*	175,6	21,1	241,3	228,9	*12,4*	235,1	28,2
12	197,2	182,5	*14,7*	189,9	22,8	252,4	248,7	*3,7*	250,6	30,1
13	212,4	218,8	*6,4*	215,6	25,9	288,2	285,9	*2,3*	287,1	34,4
14	201,8	201,8	*0,0*	201,8	24,2	255,6	264,3	*8,7*	260,0	31,2
16	170,1	168,3	*1,8*	169,2	20,3	236,7	244,1	*7,4*	240,4	28,8
17	189,9	189,9	*0,0*	189,9	22,8	245,9	245,9	*0,0*	245,9	29,5
18	173,8	169,6	*4,2*	171,7	20,6	239,0	237,2	*1,8*	238,1	28,6
19	178,8	184,8	*6,0*	181,8	21,8	260,2	250,5	*9,7*	255,4	30,6
20	167,3	171,0	*3,7*	169,2	20,3	224,3	231,2	*6,9*	227,8	27,3
21	211,5	223,0	*11,5*	217,3	26,1	265,7	269,4	*3,7*	267,6	32,1

Table 5-: Data (mU/l) for Skimmed Cow Milk samples:
Raw data, Difference between duplicates, mean and "individual" repeatability limit

4.2.3 Statistical evaluation

4.2.3.1 Determination of the assigned value: Robust mean X*

	Whole		Semi skim		Skimmed	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
X*	**196,3**	**257,0**	**199,7**	**265,6**	**184,7**	**248,1**

Table 6: Assigned value

4.2.3.2 Determination of the target standard deviation, $\hat{\sigma}$

	Whole		Semi skim		Skimmed	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
X*	196,3	257,0	199,7	265,6	184,7	248,1
Sr	8,4	11,0	8,6	11,4	7,9	10,6
SR	16,8	22,0	17,1	22,8	15,8	21,3
$\hat{\sigma}$	**15,7**	**20,6**	**16,0**	**21,3**	**14,8**	**19,9**

Table 7: Target Standard Deviation, $\hat{\sigma}$

4.2.3.3 Determination of the individual performance, *z*-scores

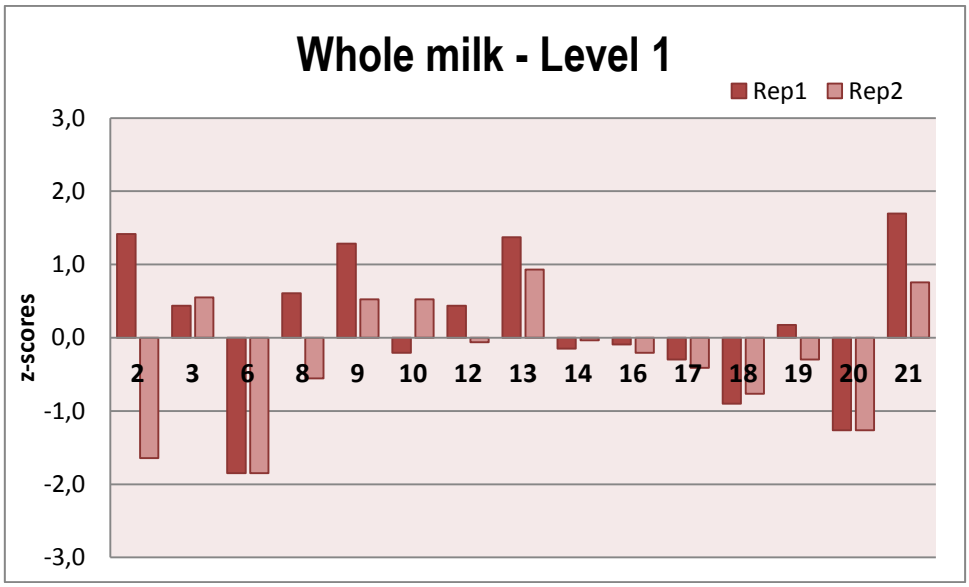
Lab. code	Whole				Semi-Skim				Skimmed			
	Level 1		Level 2		Level 1		Level 2		Level 1		Level 2	
	Rep1	*Rep2*	*Rep1*	*Rep2*	*Rep1*	*Rep2*	*Rep1*	*Rep2*	*Rep1*	*Rep2*	*Rep1*	*Rep2*
2	1,4	-1,6	0,0	-0,4	-0,7	-1,4	0,9	-1,0	-1,5	-1,5	0,0	-1,1
3	0,4	0,6	0,7	0,7	0,6	1,1	1,2	0,8	1,0	1,3	0,8	0,9
6	-1,8	-1,8	-1,0	-1,2	-0,9	-0,7	-0,4	-0,7	-0,7	-0,6	-0,5	-0,8
8	0,6	-0,6	-0,2	-0,1	-0,4	-0,6	-0,3	-0,7	-1,6	-1,9	-1,8	-1,1
9	1,3	0,5	0,8	1,0	0,5	0,8	0,8	0,6	0,7	0,3	0,8	1,0
10	-0,2	0,5	-0,4	0,4	1,1	0,9	0,2	0,6	-0,6	-0,7	-0,3	-1,0
12	0,4	-0,1	0,4	0,3	0,0	-0,2	-0,1	0,2	0,8	-0,1	0,2	0,0
13	1,4	0,9	1,6	1,3	1,4	1,5	1,8	0,5	1,9	2,3	2,0	1,9
14	-0,1	0,0	0,0	0,0	-0,3	-0,3	-0,2	-0,6	1,2	1,2	0,4	0,8
16	-0,1	-0,2	-0,1	0,2	0,2	-0,3	0,8	0,4	-1,0	-1,1	-0,6	-0,2
17	-0,3	-0,4	-0,4	-0,6	-0,5	-0,5	-0,3	-0,4	0,4	0,4	-0,1	-0,1
18	-0,9	-0,8	-0,5	-0,4	-0,6	-0,4	-0,6	-0,5	-0,7	-1,0	-0,5	-0,5
19	0,2	-0,3	0,4	0,4	0,1	-0,8	-0,4	-0,3	-0,4	0,0	0,6	0,1
20	-1,3	-1,3	-0,7	-1,1	-1,0	-1,2	-1,2	-0,9	-1,2	-0,9	-1,2	-0,8
21	1,7	0,8	-1,3	0,8	1,8	0,9	-0,5	0,6	1,8	2,6	0,9	1,1

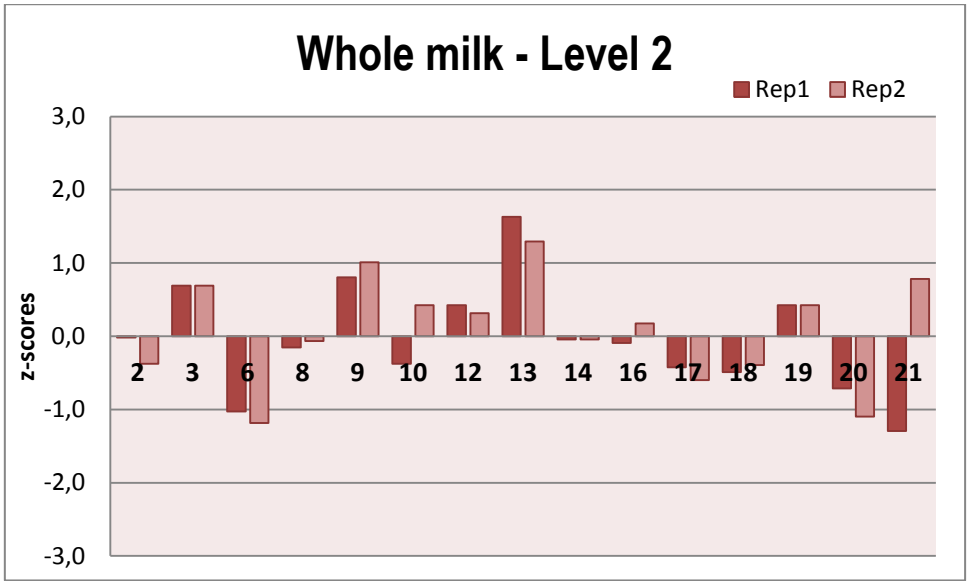
Table 8: Individual performance, z-scores

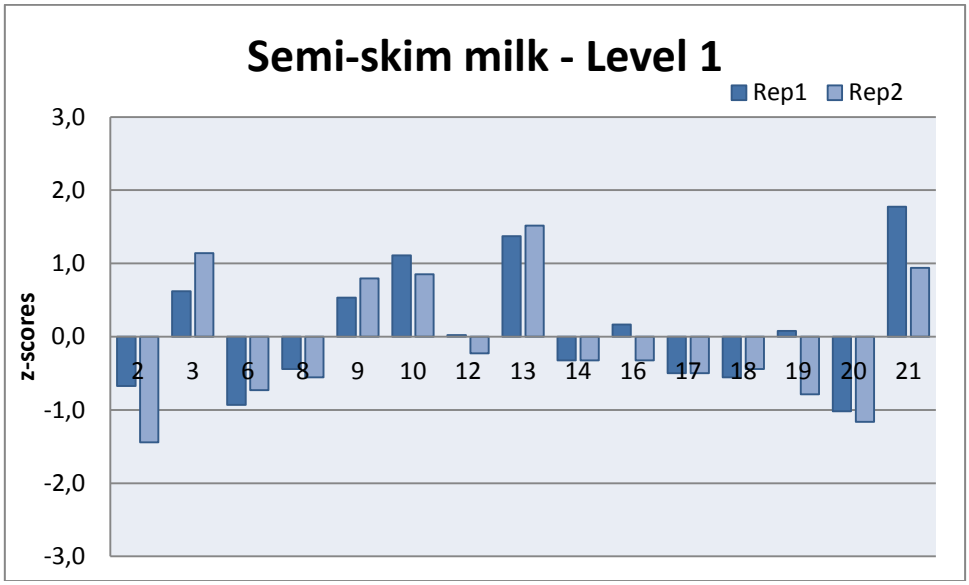
Non-repeatable

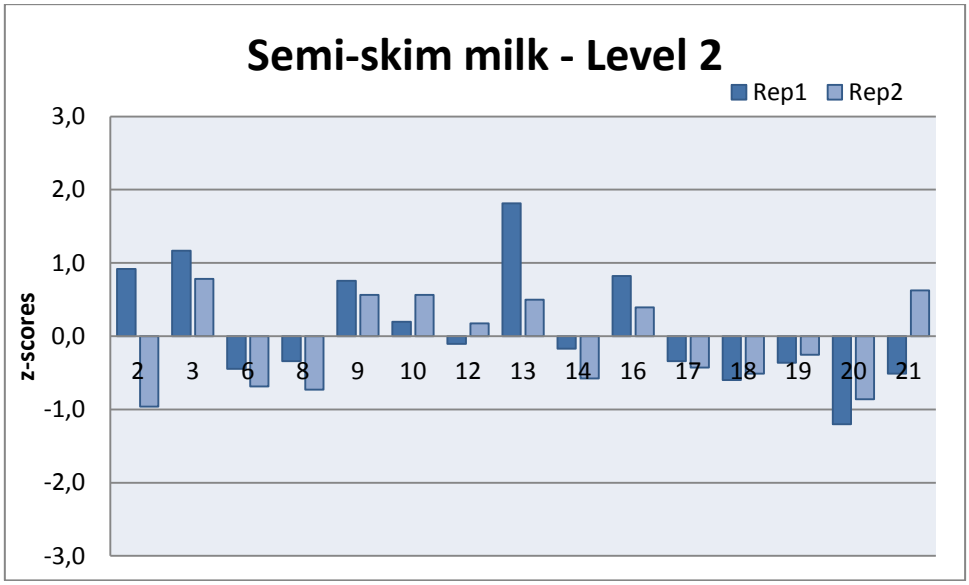
$\lvert z \rvert \leq 2,0$	**satisfactory**
$2,0 < \lvert z \rvert \leq 3,0$	**questionable**
$\lvert z \rvert > 3,0$	**unsatisfactory**

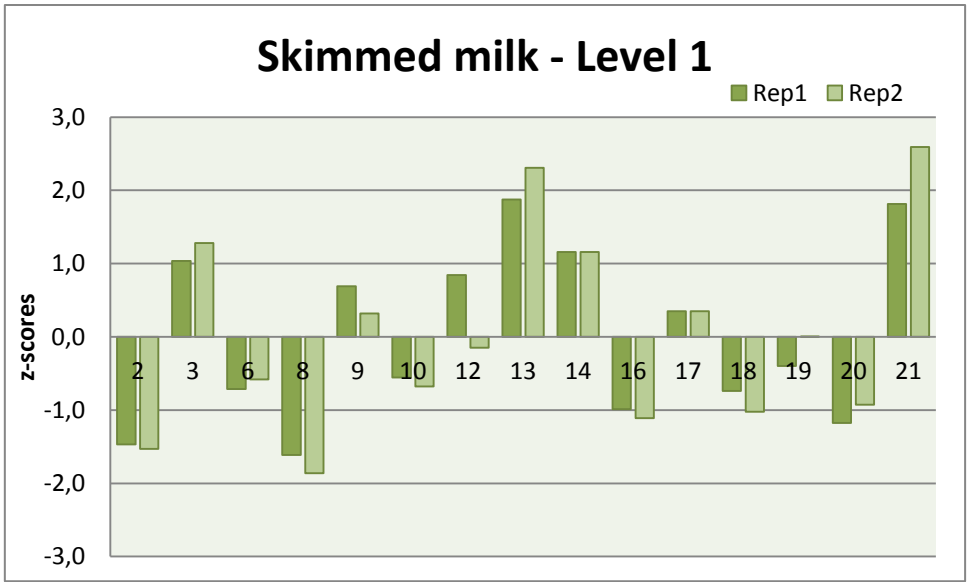
4.2.3.4 Graphical representation of z-scores

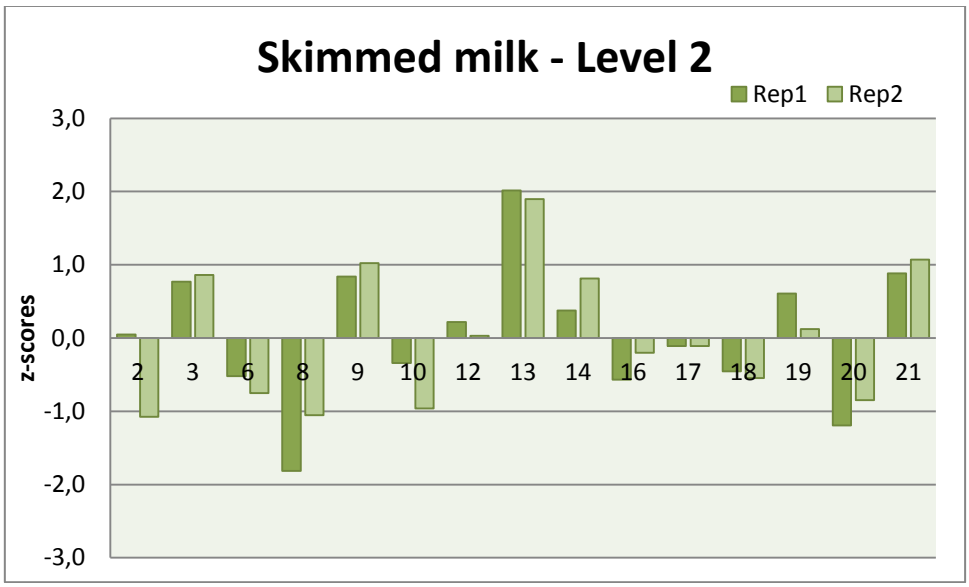












5. OVERVIEW OF RESULTS, FOLLOW-UP & CONCLUSION

Twenty one laboratories had expressed willingness to participate to the PT trial.

Laboratories 1 and 11 failed to submit results; they had not been able to participate because of unpredictable technical problems.

From the remaining 19 laboratories, four were rejected from the results' evaluation (laboratories 4, 5, 7 and 15) because of non-respect of the organizer's instructions (Table 1).
Laboratory 4 obtained a value for the daily A/D Test outside the compliance range.
Laboratory 5 did not comply with the range prescribed for the substrate.
Laboratory 7 derogated to the deadline of analysis because of difficulties encountered with technical staff.
All four laboratories were individually informed by EURL that their results were refused and were addressed a discrepancy form.

As regards laboratory 5, exchanges between EURL and NRL concluded that the discrepancy was due to the poor quality of the reagent bought through the local representative of the company producing the reagent. Performance of the NRL is not at all at the origin of the discrepancy.
EURL established contact with the mother company (USA) so that action is taken to avoid that such problems occur again.
Laboratories 1, 4 and 11 faced equipment failures and asked for technical assistance from the local representative of the manufacturer but the latter didn't succeed in servicing the instruments. EURL drew attention of the manufacturer on this aspect and urged them for action measures.
This having been said, it has nevertheless not been possible to evaluate the performance of the above mentioned laboratories through this trial.

As for laboratory 15, they neither applied the prescribed method nor respected the date of analysis. This NRL did not react to EURL's query about the discrepancies observed despite three reminders.
Same types of problems had been already recorded in previous PTs and consequently this NRL gets a very unsatisfactory evaluation.

As refers to laboratory performance evaluation according to the precision criteria, i.e. respect of the repeatability limit, three individual results did not respect repeatability limit. The laboratories concerned were sent a discrepancy form citing the observed lack of repeatability and asking for feedback on the corrective measures implemented.
Laboratory 2 had two non-repeatable results, one on whole milk level 1 and the other one on semi-skim milk level 2. Fruitful discussion between EURL and laboratory 2 pointed to the ageing instrument (that the laboratory intended to replace) as a possible cause of this failure. In the meantime the laboratory was equipped with a new instrument and the problem did not appear again so long.
Laboratory 21 had one non-repeatable result on whole milk sample, level 2. Despite the efforts of EURL and NRL to elucidate the origin of this problem, it had not been possible to identify any plausible cause.

When it comes to the individual performance of laboratories in terms of trueness (z-scores), laboratory performance is in general very satisfactory (97 %).

No unsatisfactory z-scores were detected. Only 2 questionable z-scores were recorded on skimmed milk samples, level 1. One case was assigned to laboratory 13 and the second one to laboratory 21.
No action was deemed necessary for laboratory 13 but laboratory 21, which accumulates one non-repeatability and one questionable z-score, received a warning asking them for increased surveillance so as to identify any possible cause to these problems.

Further to the evaluation of the laboratory performance, EURL seized the opportunity of this PT to compare the precision figures obtained when submitting the data of the network to a statistical evaluation according to ISO 5725-2[5] to those derived from the international validation trial given in the ISO /DIS 11816-1 (r=12% of the mean and R=24% of the mean). Repeatability and reproducibility values calculated using the network data are much better than those of the validation study for both levels and all types of milk, with one exception: skim milk level 1 (see Appendix III).
This finding is a positive conclusion to the current PT trial.

[5] ISO 5725-Part 2:1994 "Precision (accuracy and fidelity) of the results and methods of measurements"

6. APPENDIX

❖ APPENDIX I - *Participating Laboratories*

Country (City)	Laboratory name
Austria (Vienna)	AGES - INZT
Bulgaria (Sofia)	National Reference Laboratory for Milk and Milk Products
Cyprus(Nicosia)	LCFAO, Laboratory for the Control of Foods of Animal Origin
Czech Republic (Praha)	State Veterinary Institute Prague
Spain (Santander)	Laboratorio Agroalimentario de Santander
Finland (Helsinki)	Finnish Food Safety Authority Evira
France (Maisons-Alfort)	Anses, Food safety laboratory of Maisons-Alfort
Germany (Kiel)	MRI, Max Rubner-Institute
Hungary (Budapest)	National Food Chain Safety Office, Food Analytical National Reference laboratory of Food and Feed Safety Directorate
Ireland (Co. Kildare)	Dairy Science Laboratory
Italy (Brescia)	IZSLER, Reference Center for Bovine Milk Quality
Italy (Milan)	State University of Milan - DeFENS
Belgium (Melle)	ILVO T&V
Netherlands (Wageningen)	RIKILT
Northern Ireland (Belfast)	AFBI
Norway (Moss)	Eurofins Food & Agro Testing Norway AS
Poland (Pulawy)	National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin
Portugal (Lisboa)	INIAV, I.P., Instituto Nacional de Investigação Agrária e Veterinária, I.P.
Romania (Bucarest)	Institute for Hygiene and Veterinary Public Health
Slovenia (Naklo)	University of Ljubljana - National veterinary institute, Unit Kranj
Switzerland (Bern)	Agroscope Liebefeld Posieux

❖ APPENDIX II - Coding of the samples

Level	*Whole - 0*		*Whole - 1*		*Whole - 2*		*Semi skim - 0*		*Semi skim - 1*		*Semi skim - 2*		*Skim - 0*		*Skim - 1*		*Skim - 2*	
Lab. code	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*	*rep.1*	*rep.2*
1	461	42	193	605	529	494	539	468	194	134	485	419	67	325	602	265	615	214
2	167	19	204	260	159	606	526	477	240	568	81	592	45	472	43	397	150	263
3	175	189	229	502	94	155	6	503	272	310	491	557	173	312	344	501	77	645
4	556	323	320	336	572	441	370	233	17	1	235	475	253	2	546	5	412	57
5	490	377	626	91	409	531	142	388	488	165	641	518	108	470	447	182	379	431
6	614	599	47	78	519	410	545	484	528	119	476	399	115	355	102	98	532	333
7	359	652	266	443	627	563	296	495	69	163	466	398	259	324	215	224	634	570
8	376	304	514	219	456	390	136	487	357	603	244	249	623	540	480	512	387	493
9	608	492	122	582	31	625	583	303	299	644	613	226	62	195	588	439	246	328
10	657	654	168	624	489	574	648	353	371	44	533	286	23	156	302	107	508	415
11	318	483	402	380	330	550	298	593	58	70	109	639	90	12	236	346	255	59
12	176	131	144	280	213	552	148	300	424	284	571	436	200	434	389	640	329	643
13	21	22	567	186	418	384	421	140	311	375	524	392	354	33	381	64	587	84
14	88	607	218	93	85	631	506	373	365	146	126	543	600	327	394	516	651	500
15	308	34	429	32	196	444	403	305	417	349	547	164	341	87	617	515	347	141
16	82	210	248	517	35	542	435	564	317	612	356	374	342	247	432	258	309	110
17	609	63	616	169	428	400	509	465	611	18	254	536	350	181	38	345	11	459
18	301	499	569	289	135	276	65	331	453	326	478	199	321	209	225	589	96	463
19	125	351	630	206	121	143	586	50	86	222	261	522	73	228	27	584	227	621
20	198	192	581	553	129	580	335	25	270	566	139	269	16	212	420	504	538	29
21	573	123	264	360	649	294	220	423	507	203	287	180	158	268	653	52	469	534

❖ APPENDIX III: Comparison of precision data of the network with precision data of ISO/DIS 11816-1.

Type of Milk	Level	M (mU/l)	r network	r % $(r_{network}/_{M} \times 100)$
Whole	1	195,8	16,8	9%
	2	257,9	12,1	5%
Semi skim	1	199,7	13,8	7%
	2	265,8	30,2	11%
Skim	1	184,8	11,7	6%
	2	248,8	17,9	7%

< 12 % *(ISO/DIS)*

Type of Milk	Level	M	R network	R % $(R_{network}/_{M} \times 100)$
Whole	1	195,8	40,0	20%
	2	257,9	41,0	16%
Semi skim	1	199,7	39,2	20%
	2	265,8	43,4	16%
Skim	1	184,8	51,9	**28%**
	2	248,8	51,9	21%

< 24 % *(ISO/DIS)*

→ **< 24 %** *(ISO/DIS)*

M, r network and R network are the mean and precision figures obtained when submitting the data of the network to a statistical evaluation according to ISO 5725-2[6]

[6] ISO 5725-Part 2:1994 "Precision (accuracy and fidelity) of the results and methods of measurements"