
Evaluation Report

Evaluation of the Ekomilk Horizon Unlimited Instrument (EHU) for the determination of Fat, Protein and Somatic cell in raw cow milk

According to
ISO 8196-3 | IDF 128-3, ICAR Section 12 document



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1. INTRODUCTION

1.1- OBJECTIVES

ICAR requested ACTALIA Cecalait to conduct an evaluation for the instrument EkoMilk Horizon Limited (EHU) for the determination of fat, true protein contents and somatic cell in fresh raw cow milk.

1.2- THE INSTRUMENT

The milk analyser Ekomilk Horizon Unlimited (EHU) is designed for fast and cost effective control of milk quality and milk composition via Somatic Cell Count (SCC), Fat, Protein, conductivity, Lactose, Freezing Point and added water to milk (AWM) measurement in dairy farms and dairy enterprise.

The instrument is manufactured by EKOMILK Horizon Ltd in Bulgaria and use an ultrasonic principle (ultrasonic sensor) for milk composition and an automated CMT principle for SCC.

The instrument is dedicated to **fresh raw cow milk (<12 h after milking) without any preservative** and the average time for measurement is approximately 1 min (for 1 test). Operating procedure for SCC and milk parameters calibration are available and defined by the manufacturer.

The measuring parameters, range and typical accuracy proposed by the manufacturer are the following:

Measuring Parameters – range and typical accuracy

Somatic Cell Count per 1 ml	80 000 – approx. 15 000 000 \pm 8%
Operating temperature	from 15 °C to 35°C
Average time per 1 measurement	1 min depending on average SCC level and measuring mode
Fat	from 0,5% to 12% with accuracy \pm 0,1%
Solids nonfat (SNF)	from 6% to 12% with accuracy \pm 0,2%
Milk density (CLR)	from 1,0200 to 1,0400 g/cm ³ \pm 0,0005 g/cm ³
Protein	from 1% to 9% with accuracy \pm 0,2%
Lactose	From 0.5% to 7% with accuracy \pm 0,2%
Freezing Point	from 0 to -1.000 °C with accuracy \pm 0,015°C
Added water to milk	from 0% to 60% with accuracy \pm 5%

Figure 1: Measuring parameters Ekomilk Horizon Unlimited

The two instruments were installed in a temperature controlled room (20-23°C – air-conditioning), without direct sunlight. The installation procedure was performed by ACTALIA Cecalait according to the manufacturer instructions.

1.3- CONSUMABLES

The necessary consumables (provided by the manufacturer) were:

- Ekoprim working solution (to reconstitute from powder) for SCC measurement
- Ekoday and Ekoweeek working solutions (to reconstitute) as cleaning agents

1.4- THE TESTS

The former evaluation protocol was built in accordance with the standards ISO 8196-3 | IDF 128-3 and the ICAR section 12 document. After many preliminary tests on different type of samples (freezed samples, recombined raw milk samples, diluted samples...), the protocol have been finally adapted according the possibility of the instrument and is the following :

Phase I :

- Short term (daily) reproducibility (on 2 instruments) for fat, protein and Somatic cell,
- Mid term reproducibility (6 weeks on 1 instrument) for fat, protein and somatic cell,
- Carry over for fat and somatic cell (on 1 instrument).

Phase II :

- Repeatability and accuracy (against an ICAR certified instrument) on (at least) 200 individual fresh raw cow milk samples from 5 herds on 2 instruments.

Operating conditions for the evaluation

- Calibration
 - o The calibration for fat and protein have been made according manufacturer instructions (adjustment of bias using 5 individual fresh milk samples)
 - o The calibration for SCC has not been adjusted (not possible regarding the different milks needed for such operation), so we used the “default” calibration.
- The samples have been warm up in a **waterbath at 25°C** and gently mixed manually by rotating the vials.
- All the cleaning procedures defined by the manufacturer (included manual cleaning funnels and flasks for SCC) have been respected during the tests, **we added a daily systematic extra cleaning process** (3 cycles water / 5 cycles Ekomilk / 3 cycles water) as a startup sequence before analyse (experience from preliminary testings)

2. EVALUATION OF SHORT TERM (DAILY) REPRODUCIBILITY

2.1- Procedure

The evaluation of the short term reproducibility on fat & protein contents and somatic cell have been made by analysis of 2 fresh raw cow milks in duplicate, every 20 minutes to obtain 15 sequences. This test has been performed on the 2 instruments.

2.2- Results

The following tables¹ present the results obtained:

		Instrument 1		Instrument 2	
		Milk 1	Milk 2	Milk 1	Milk 2
Fat (g/100g)	M	3.48	3.91	3.46	3.89
	Sr	0.011	0.018	0.007	0.005
	SR	0.026	0.037	0.015	0.019
	r	0.031	0.049	0.021	0.014
	R	0.073	0.102	0.042	0.052

Table 1: Ekomilk - Short term reproducibility for fat

		Instrument 1		Instrument 2	
		Milk 1	Milk 2	Milk 1	Milk 2
Protein (g/100g)	M	3.27	3.34	3.25	3.32
	Sr	0.008	0.012	0.005	0.005
	SR	0.016	0.025	0.008	0.012
	r	0.021	0.034	0.013	0.014
	R	0.045	0.070	0.023	0.033

Table 2: Ekomilk - Short term reproducibility for protein

		Instrument 1		Instrument 2	
		Milk 1	Milk 2	Milk 1	Milk 2
Somatic cell (x 10 ³ /ml)	M	695	221	755	236
	Sr	36	6	39	8
	Sr(%)	5.1	2.8	5.2	3.4
	SR	44	7	44	8
	SR(%)	6.3	3.1	5.8	3.4

Table 3: Ekomilk - Short term reproducibility for somatic cell

2.3- Conclusion

The values of standard deviation of reproducibility for fat, protein and somatic cell are below to the limits defined in the ICAR Section 12 document: 0,04g/100 g for fat and protein and 10 % for somatic cell (full range).

3. EVALUATION OF MID TERM REPRODUCIBILITY

¹ M: mean; Sr and SR: absolute standard deviations of repeatability and reproducibility; r and R: maximum deviation of repeatability and reproducibility in 95 % of cases.

3.1- Procedure

The evaluation of the mid-term reproducibility on fat, protein and somatic cell has been made by analysis of 2 vials of 2 freezed (-80°C) raw milk samples in duplicate 2 or 3 times per week for 6 weeks on 1 instrument. Before analysis, the freezed samples have been defreezed in a waterbath at 25°C for 30 min and gently mixed.

3.2- Results

The following tables² present the results obtained:

		Instrument 1	
		Milk 1	Milk 2
Fat (g/100g)	M	3.38	3.83
	Sr	0.028	0.021
	SR	0.054	0.046
	r	0.077	0.059
	R	0.149	0.129

Table 4 : Ekomilk - Mid term reproducibility for fat

		Instrument 1	
		Milk 1	Milk 2
Protein (g/100g)	M	3.25	3.32
	Sr	0.013	0.013
	SR	0.034	0.039
	r	0.035	0.035
	R	0.094	0.109

Table 5: Ekomilk - Mid term reproducibility for protein

		Instrument 1	
		Milk 1	Milk 2
Somatic cell (x 10³/ml)	M	244	139
	Sr	8	4
	Sr(%)	3.2	2.7
	SR	27	11
	SR(%)	11.0	7.9

Table 6 : Ekomilk - Mid term reproducibility for somatic cell

² M: mean; Sr and SR (Sr% and SR%): absolute (and relative) standard deviations of repeatability and reproducibility; r and R: maximum deviation of repeatability and reproducibility in 95 % of cases.

3.3- Conclusion

Considering the reference document don't have any prescribed limits for Mid-term reproducibility tests (limit only for Daily reproducibility), we can observe reproducibility values obtained are nevertheless in the limit of the Daily reproducibility test for protein (respectively 0.034 & 0.039 vs 0.04 g/100 g) and closed to the limit for fat (0.054 & 0.046 vs 0.04g/100 g). For somatic cell, the results obtained are closed to the SR % limit of 10 % (respectively 11.0 and 7.9 % for both instruments).

4. EVALUATION OF CARRY OVER EFFECT

4.1- Procedure

The evaluation of the carry over effect on fat /protein and somatic cell has been made by analysis of 10 sequences « Milk - Milk - Blank milk - Blank milk ».

- For fat and protein, the « milk » was a fresh raw cow milk and the « blank milk » was a fresh milk diluted (1/8) (validation of this possibility in the preliminary tests)
- For somatic cell, the « milk » was a fresh raw cow milk at around 600 x10³ somatic cell / ml and the « blank milk » was a fresh raw cow milk at around 100 x10³ somatic cell / ml.

4.2- Results

The following table present the results obtained:

Criteria	Mean H	Mean L	Calculated C (H/L) %
Fat (g/100 g)	3.46	0.38	-0.03 %
Protein (g/100 g)	3.39	0.55	-0.13 %
Somatic cell (x 10 ³ /ml)	611	97	3.0 %

Table 7 : Ekomilk Carry over tests

4.3- Conclusion

The calculated carry-over effect C(H/L) for all the criteria tested (fat, protein and somatic cell) are lower than the limits defined in the ICAR Section 12 document : At line instruments (respectively 2 % for fat and protein and 4 % for somatic cell).

5. EVALUATION OF THE REPEATABILITY AND ACCURACY

5.1- Samples

The tests were performed on 228 fresh raw cow milk samples collected in 5 herds by a regional DHI organization (East of France).

5.2- Repeatability

5.2-1. Procedure

The repeatability of the instrument was evaluated by successive analyse of the samples on the 2 instruments.

5.2-2. Results

The following table³ presents the results obtained:

Instrument 1	n	Min	max	M	S _r	S _r %	r
Fat (g/100g)	228	2.23	5.33	3.62	0.003		0.009
protein (g/100g)	228	3.01	3.56	3.31	0.002		0.006
Somatic cell (x10 ³ /ml)	225	68	1064	231	6.3	2.7	77

Table 8 : Ekomilk repeatability criteria for fat, protein and somatic cell in raw cow milk samples

Instrument 2	n	Min	max	M	S _r	S _r %	r
Fat (g/100g)	228	2.23	5.36	3.63	0.004		0.011
protein (g/100g)	228	3.02	3.56	3.31	0.003		0.008
Somatic cell (x10 ³ /ml)	226	95	968	260	14.4	5.5	40

Table 9 : Ekomilk repeatability criteria for fat, protein and somatic cell in raw cow milk samples

5.2-3. Conclusion

For fat and true protein, the standard deviation of repeatability are in accordance with the recommendations of the ICAR section 12 document ($S_r < 0,028$ g/100g).

For somatic cell, the relative standard deviation of repeatability for the full range is below the limit of 8 % prescribed in the ICAR section 12 document.

5.3- Accuracy

³ N : number of samples ; Min max : min and max values ; M: mean; S_r and S_r%: absolute (and relative) standard deviations of repeatability; r: maximum deviation of repeatability in 95 % of cases.

5.3-1. Procedure

The accuracy of the instrument was evaluated by using of the mean of the instrument results from the repeatability test compared to a single analyse using an ICAR certified instrument:

- For milk composition : IR instrument Foss 7 RM calibrated against chemical reference methods for fat (Gerber – ISO 19662 | IDF238) and true protein (Amido black method – NF V 04 216)
- For somatic cell : flow cytometry instrument Foss 7 DC calibrated using SRM traceable to EU CRM

5.3-2. Results

The following tables ⁴ and figures present the results obtained:

INS 1	Fat* (g/100 g)	Protein (g/100 g)	Somatic cell Full range (x10 ³ /ml)	Somatic cell 0-500 x10 ³ / ml (x10 ³ /ml)
N	224	228	224	218
min	2.23	3.01	68	68
Max	4.87	3.56	781	659
Y	3.53	3.31	100	76
d	+0.09	+0.11	+75	+86
S_{y,x}	0.103	0.129		
S_{y,x} %			38.6	19.6

Table 10 : Ekomilk 1 accuracy criteria for fat, protein and somatic cell in raw cow milk samples

INS 2	Fat (g/100 g)	Protein (g/100 g)	Somatic cell Full range (x10 ³ /ml)	Somatic cell 0-500 x10 ³ / ml (x10 ³ /ml)
N	224*	228	224	218
min	2.23	3.02	95	95
Max	4.88	3.56	968	709
Y	3.53	3.31	100	76
d	+0.10	+0.11	+97	+107
S_{y,x}	0.103	0.127		
S_{y,x} %			35.7	16.9

Table 11: Ekomilk 2 accuracy criteria for fat, protein and somatic cell in raw cow milk samples

*4 samples detected as outliers for both instrument for fat

⁴ n, min, max: number of results, minimum and maximum values; Y: mean results using the reference method; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x, Sy,x%: absolute and relative residual standard deviation.

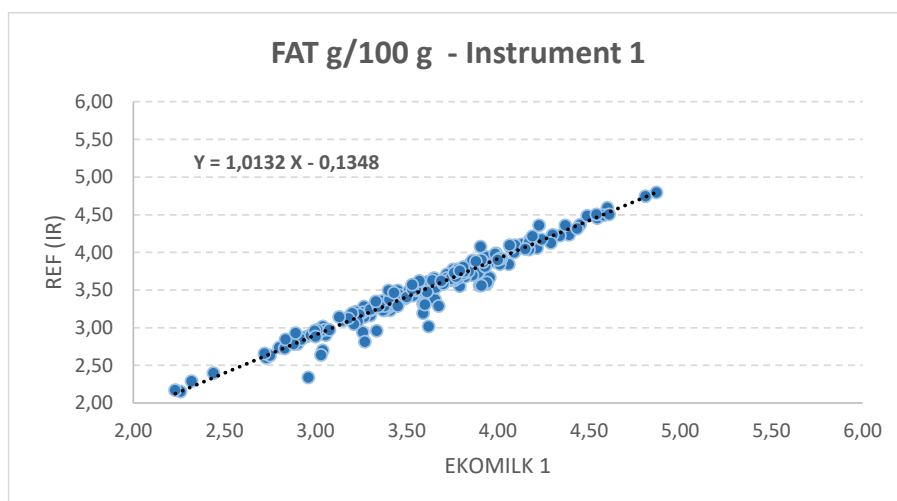


Figure 2: Relation between EkoMilk 1 and reference results for fat on raw cow milk

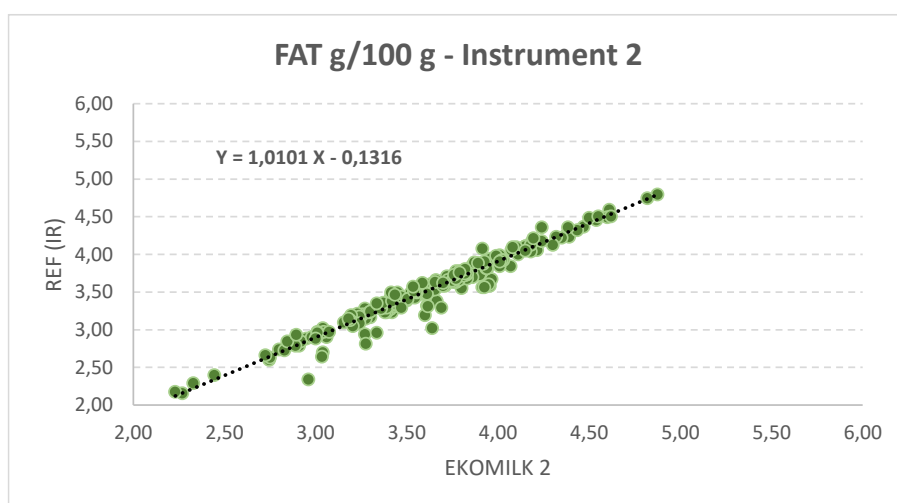


Figure 3: Relation between EkoMilk 2 and reference results for fat on raw cow milk

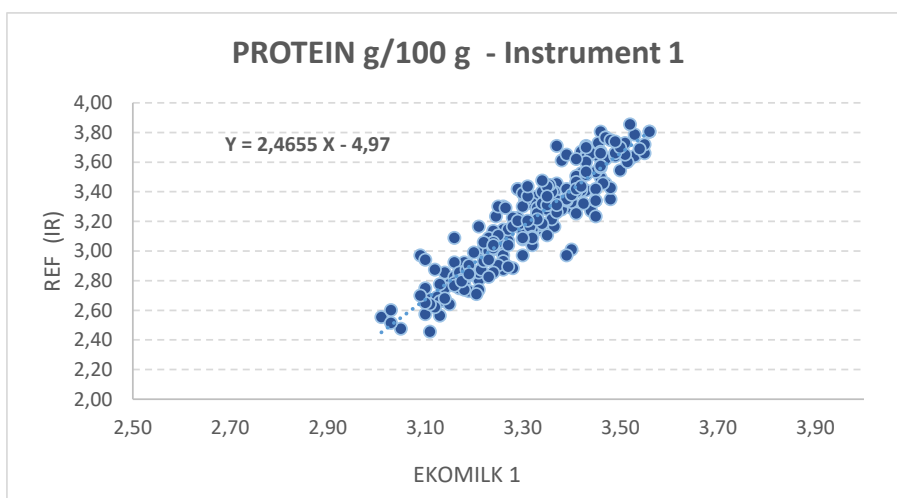


Figure 4 : Relation between Ekomilk 1 and reference results for protein on raw cow milk

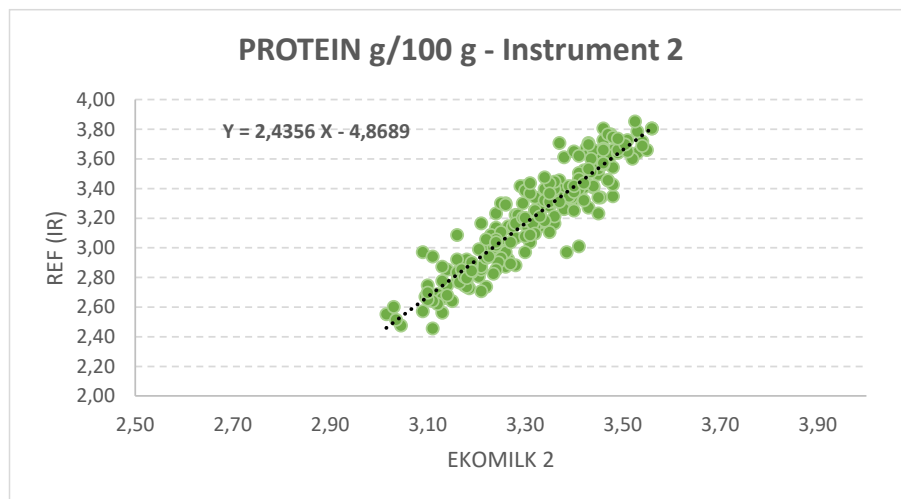


Figure 5 : Relation between Ekomilk 2 and reference results for protein on raw cow milk

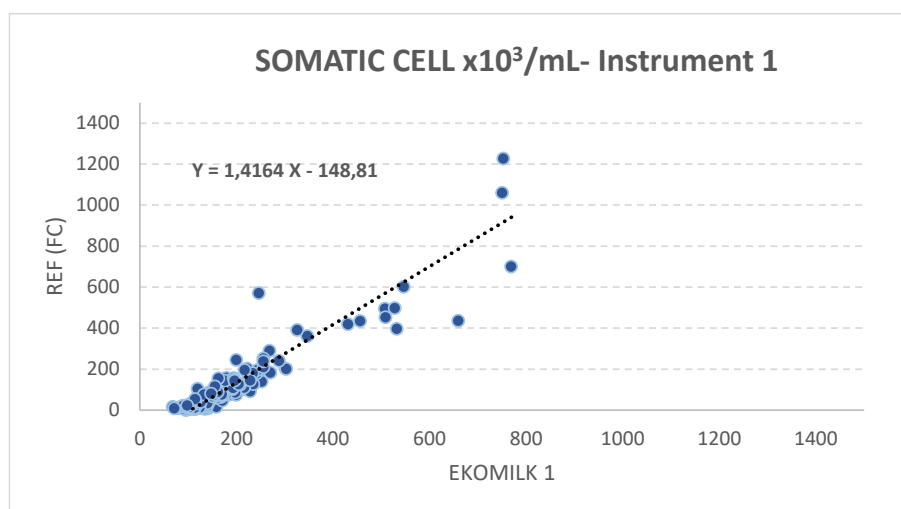


Figure 6 : Relation between Ekomilk 1 and reference results for Somatic cell on raw cow milk

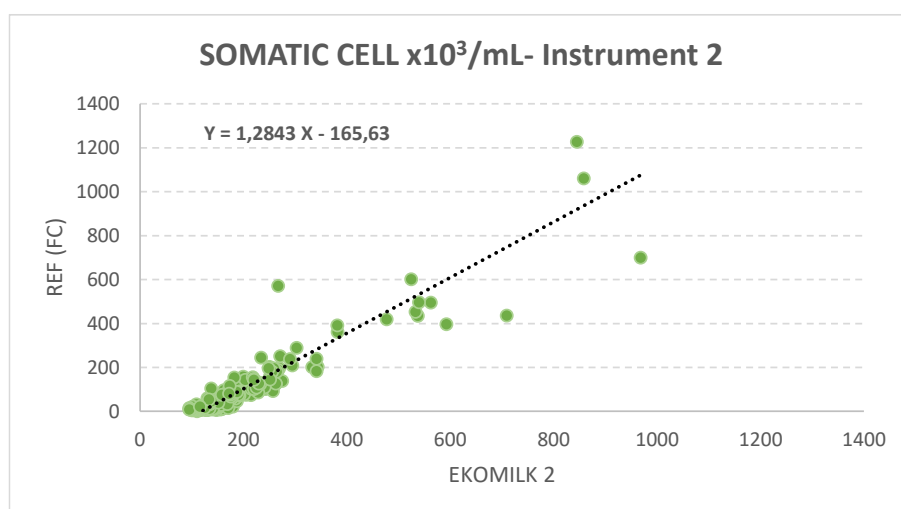


Figure 7 : Relation between Ekomilk 2 and reference results for Somatic cell on raw cow milk

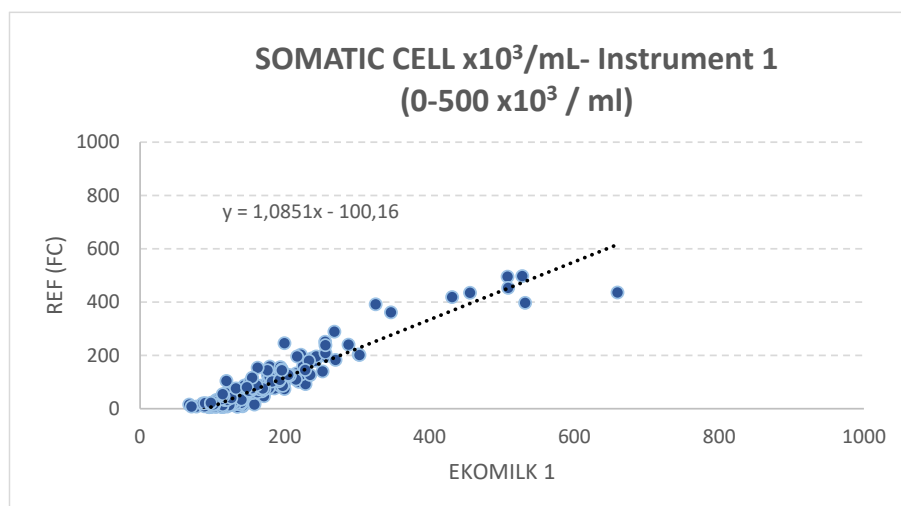


Figure 8 : Relation between Ekomilk 1 and reference results for Somatic cell on raw cow milk (0-500 x 10³ / ml)

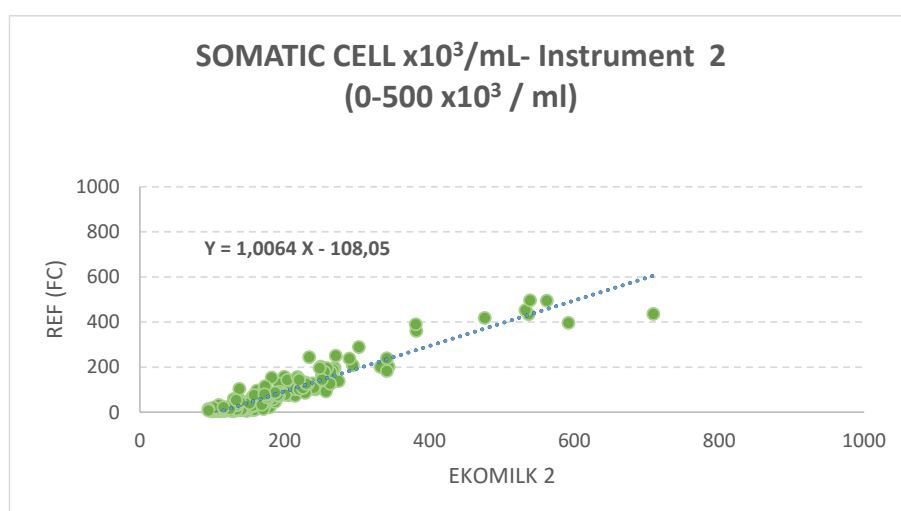


Figure 9 : Relation between Ekomilk 2 and reference results for Somatic cell on raw cow milk (0-500 x 10³ / ml)

5.3-3. Conclusion

For fat prediction, the residual standard deviation for both instruments are below the limit of 0.12 g/100 g described in the ICAR section 12 document. The slope for both instruments are very closed to 1.00.

For protein prediction, the residual standard deviation for both instruments are very closed to the limit : respectively 0.129 and 0.127 for both instruments vs 0.12 g/100 g). Considering the narrow range in protein concentration of the milk tested, the slopes obtained from RLS calculation should not be taken into account for evaluation.

For somatic cell, the relative residual standard deviation obtained on both instruments on full range are significantly above the limit of 20 % (respectively 38.6 % and 35.7 % vs 20 %). If we select the concentration range from 0 to 500 x 10³ somatic cell /ml for evaluation of the accuracy of the instrument, the relative residual standard deviation are in the limit of 20 % for both instruments (respectively 19.6 % and 16.9 %). In addition, the slope of the linear regression is very close to 1.00 (respectively 1.08 and 1.00) using the “default” calibration of the instrument.

6. CONCLUSION

The conclusions on the different tests conducted during this evaluation are the following:

- The short term reproducibility of the instrument have been tested and prescribe to the ICAR Section 12 document limit for such type of instrument (at Line instrument).
- The results obtained in the mid-term reproducibility test on 6 weeks show a good stability of the instrument using freezed samples, even if the results obtained are a little bit above the limit for daily reproducibility.
- The test on carry over have been successful for all the criteria tested
- The repeatability values obtained by analysing more than 200 fresh raw cow samples are in the limits prescribed in the ICAR Section 12 document for such type of instrument.
- Concerning the accuracy, the performance obtained for composition criteria (fat and protein) are in the limit of 0.12g / 100 g (Sy,x) for fat and very closed to this limit for protein.

For somatic cell, the performance obtained on the full range don't fulfil the requirements of 20 % (Sy,x %) of the ICAR document, but if we limit the range from 0 to 500 x 10³ somatic cell/ml, the performance of the instrument is better and fulfil the ICAR requirements for both instruments.

Philippe Trossat, 04/07/2025

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