THE GLOBAL STANDARD
FOR LIVESTOCK DATA

ICAR CERTIFICATION OF MILK ANALYSER

Company name: Foss Analytical A/S
Instrument name: Fossomatic™ 7
Milk species: Cow milk

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Preface

Fossomatic™ 7 was introduced onto the market at the end of 2016. It is an automatic, dedicated fluoro-optoelectronic instrument, based on flow cytometry, used for a rapid determination of somatic cell count in raw milk.

Fossomatic ™ 7 can measure the total somatic cell count in fresh or preserved raw milk. The instrument is mainly applied in central milk testing and dairy laboratories for payment and Dairy Herd Improvement (DHI) analyses. In this certification for ICAR DHI purposes, its performance has been evaluated in two phases: phase I, considering the results from single laboratory testing obtained during a Microval certification process and phase II with the organisation of an interlaboratory study for total somatic cell count in cow milk. For this instrument, a specific robustness test has not been performed but this will be done in the coming years and provided in connection with the certification renewal. However, the robustness test is not mandatory for the ICAR certification..

The performances will be evaluated against criteria listed in:

- ICAR protocol “Procedure 1 of Section 12 of ICAR Guidelines – Protocols for Evaluation of Milk Analyses for ICAR Approval”, which in turn is aligned with
- ISO 8196-3|IDF 128-3:2009 – Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis and
- ISO 13366-2|IDF 148-2 Milk — Enumeration of somatic cells — Part 2: Guidance on the operation of fluoro-opto-electronic counters

ISO 8196-3|IDF 128-3:2009 is currently under revision and the next edition will report limits to validate a new instrument against the previous generation. These limits will be considered as informative in this report.

Summary

Principle

Fossomatic™ 7 is an automatic, dedicated fluoro-optoelectronic instrument, based on flow cytometry, used for a rapid determination of the somatic cell count in raw milk.

Scope

The scope of this validation is total somatic cell counting in raw cow milk.

Data evaluated

Microval validation report executed at Qlip (Phase I) (2017)

Interlaboratory study (ILS) and statistical elaboration by Qlip (NL) in 2019-2020.

Table. Performance summary table for Fossomatic ™ 7

			*10³cells/ml	r%		Limit r %
		Low	90	11		28
		Medium	508	5		11
		High	1520	3		5
				Single cow milk sample	Bulk milk sample	
Repeatability Phase I	r		*10³cells/ml	r %	r %	Limit r %
		Level 1	50-200	7	9	17
		Level 2	201-400	6	6	14
		Level 3	401-650	6	5	11
		Level 4	650-1000	4	4	8
		Level 5	1000-1500	4	2	8
			Overall	5,4	5,2	11
			*10³cells/ml	R_{intra} %		Limit R_{intra} %
Intralaboratory reproducibility	R_{intra}	Low	90	20		**20**
		Medium	510	10		**14**
		High	1520	6		**11**
			*10³cells/ml	$CO_{H/L}$ %	$CO_{L/H}$ %	
Carry over Phase I	CO $_{H/L}$	Low	500	0,14	0,48	Limit CO $_{H/L}$ ≤ 2%
		Medium	1.000	0,07	0,14	
		High	3.000	0,05	0,32	
		*10³cells/ml		%		
Linearity Phase I	r $_{linearity}$	0-10.000		1,8		Limit r linearity ≤ 2%
		100-1.500		0,8		
		*10³cells/ml		%		
Accuracy Phase I	S_{yx} FC 7 vs FC Phase I	50-2.000	Single cows milk	5,8		Limit S_{yx} ≤ 8%
			Herd cows milk	4,1		

		Level of concentration	r	r	Limit ICAR	Limit ISO 13366-2/IDF 148-2	
		*10^{3}cells/ml	*10^{3}cells/ml	%		*10^{3}cells/ml	%
Repeatability (R) Interlaboratory Study (ILS) Phase II	Sample 1	176	13	8		29	16
	Sample 2	134	14	11		23	17
	Sample 3	307	16	5		46	15
	Sample 4	377	18	5		47	12
	Sample 5	523	20	4		55	11
	Sample 6	1031	17	2		86	8
	Sample 7	1306	40	3		110	8
	Sample 8	1438	35	2		121	8
	Sample 9	229	11	5		35	15
	Sample 10	277	21	7		40	14
	Overall			5	11		13

		Level of concentration	R	R	Limit ICAR	Limit ISO 13366-2/IDF 148-2	
		*10^{3}cells/ml	*10^{3}cells/ml	%		*10^{3}cells/ml	%
Reproducibility Interlaboratory Study (ILS) Phase II	Sample 1	176	22	12		44	27
	Sample 2	134	14	11		34	28
	Sample 3	307	42	14		68	24
	Sample 4	377	38	10		79	23
	Sample 5	523	63	12		99	21
	Sample 6	1031	147	14		162	17
	Sample 7	1306	158	12		192	16
	Sample 8	1438	97	7		205	16
	Sample 9	229	18	8		54	26
	Sample 10	277	33	12		63	25
	Overall			11			22

Note: In red are the proposed values for the next version of the ICAR protocol and ISO 8196-3|IDF128-3.

Final Conclusion

The outcome from the Microval validation and the results of an interlaboratory study (ILS) organised by Qlip provided a good illustration of the performance of the instrument. The adequate instrument performance in phase I was confirmed in phase II. The instrument complies with all criteria and limits as defined in Procedure 1 of Section 12 of the ICAR Guidelines – Protocol for Evaluation of Milk Analysers for ICAR Approval, which, in turn, is aligned with ISO 8196-3|IDF 128-3:2009 – Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis and EN ISO 13366-2|IDF 148-2:2006 Milk - Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-optoelectronic counters for all the criteria tested.

Based on the results of our investigations as described in this report, the Fossomatic ™ 7 can be granted the ICAR certificate for milk analysers.

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1 Introduction

ICAR certification for milk analysers is intended to serve as a standardized process for certification of milk analysers and to describe instrument performance according to ICAR international guidelines for DHI analyses in cow milk.

Fossomatic™ 7 is a new generation of Fossomatic instruments for somatic cell counting in raw milk.

FOSS launched the Fossomatic™ 7 in October 2016.

Fossomatic™ FC was granted the ICAR grandfather exception in February 2020. Fossomatic™ 7 accuracy was determined against Fossomatic™ FC during the Microval validation study as conducted by Qlip in 2017. For phase I of the certification process, ICAR made use of the information and data provided with this earlier conducted Microval certification, see https://microval.org/en/issued-certificates/. Qlip was subcontracted as expert and accredited laboratory to prepare the samples used for the Inter Laboratory Study (ILS) and perform statistical analyses of all results obtained.

The tests performed in phase I, phase II and in the ILS study are robust and independent tools to evaluate the performance of the Fossomatic™ 7.

2 Company name and instrument under evaluation

Manufacturer: FOSS Analytical A/S

Address: Nils Foss Allé 1, 3400 Hillerød, Denmark

Instrument: Fossomatic™ 7

3 Data evaluated by ICAR

Microval validation report (Phase I).

Interlaboratory study and statistical elaboration by Qlip (NL) in 2019-2020 (Phase II).

4 Instrument principle

Fossomatic™ 7 is a fully automated flow cytometer for the rapid enumeration of somatic cells in raw milk. The working principle of the instrument is based on colouring the somatic cells with a fluorescent dye - ethidium bromide - after which they are counted electronically. In the flow cytometer, the mixture of milk and staining solution is surrounded by a sheath liquid and passed through a flow cell. In the flow cell, the stained somatic cells are exposed to light of a specific wavelength. The cells emit fluorescent light pulses at a different wavelength, and the pulses are amplified and recorded by a photo detector, identified by an algorithm, multiplied by the working factor and displayed as a somatic cell count in thousands per milliliter. The design of the flow cell ensures that single cells are separately counted.

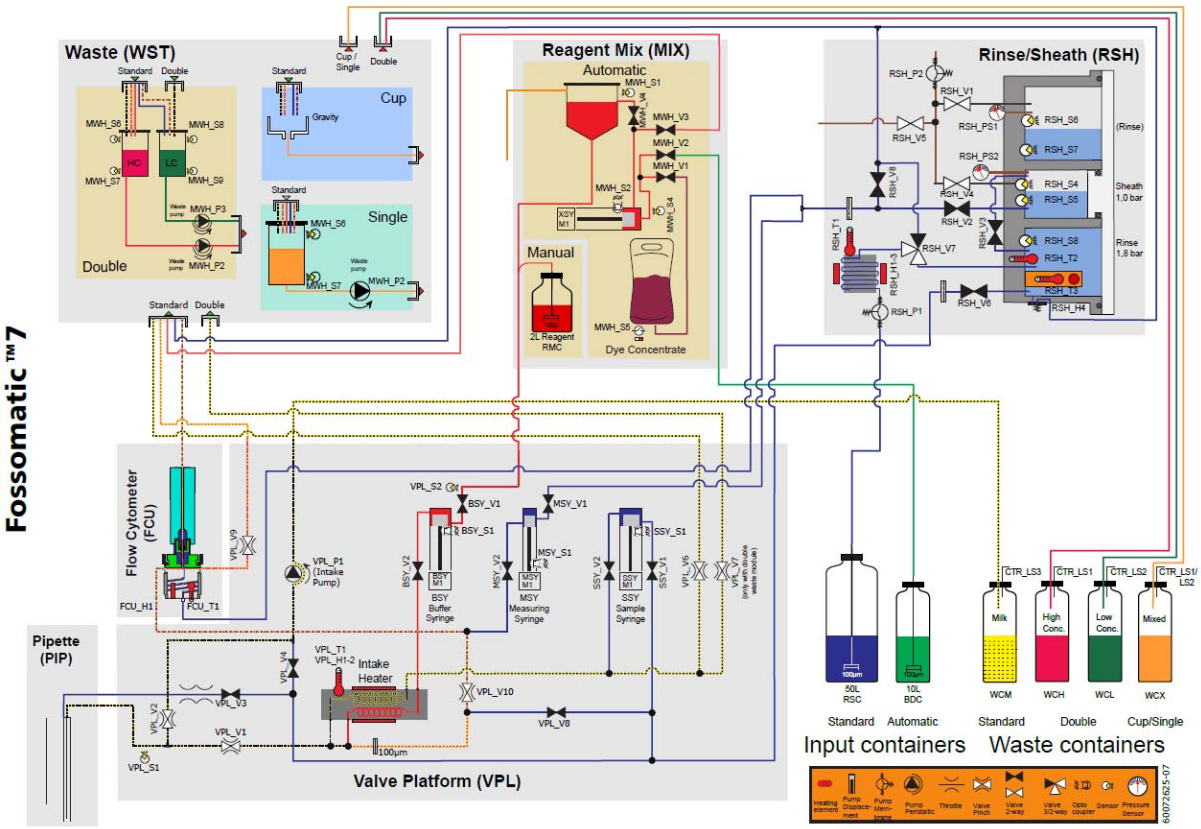

Figure 1. Scheme for measurement principle Fossomatic™ 7 (source Fossomatic™ 7 / 7 User Manual 6007 1937 / Rev. 4)

Figure 2. Fossomatic™ 7.

5 ICAR evaluation criteria

The Fossomatic™ 7 was evaluated based on the criteria and against the limits defined in Procedure 1 of Section 12 of the ICAR Guidelines – Protocols for Evaluation of Milk Analyses for ICAR Approval.

Fossomatic™ 7 accuracy was evaluated through result comparison with Fossomatic™ FC, which already obtained the ICAR Grandfather exception. Fossomatic™ FC accuracy was evaluated by comparing instrument results with results obtained through the microscopy based reference method (ISO 13366-1|IDF 148-1). Results of Fossomatic FC were found to be at least equivalent with the results of the reference method. In the Microval validation, Fossomatic™ FC was used as anchor method to evaluate the Fossomatic™ 7.

Limits for this comparison (new instrument Fossomatic™ 7 against old instrument Fossomatic™ FC) are not yet in the current ICAR protocol, but are proposed with the underway revision of ISO 8196-3|IDF 128-3. These proposed values are indicated in red in Table 1.

Table 1. ICAR evaluation criteria and limits

Measurand		**Criteria**	**Criteria limits**
(units)	**SCC**		**SCC**
	1000 cells/ml	**Intra laboratory reproducibility (sR intra)**	**%**
Range, DLrange - Overall	0 to 2 000	Relative standard deviation of reproducibility intra, sRintra % - Overall	5
- Low (L)	0 to 100	- Low (L)	7
- Medium (M)	100 to 1 000	- Medium (M)	5
- High (H)	>1 000	- High (H)	4
Carry-over ratio limit, LC		**Accuracy (syx) (1)**	
	2%	**Comparison of alternative against reference method**	
Sequence number, NC	20	Individual animal milk samples	
Minimum range, DLtest	500	Relative standard deviation of s_{yx} %	10 %
Linearity: ratio limit, De/DL		Number of individual animal milk samples, Na	100
	2%	Herd bulk milk samples'	
Replicate number for linearity, NL	8	Relative standard deviation of s_{yx} %	10 %
Maximun range, DLtest	2 000	Number of herds, Nh1	5
Repeatability (sr)		Number of herd bulk milk samples'	60
Relative standard deviation of repeatability , sr%		**Accuracy (syx) (2)**	
- Overall	4 %		
- Low (L)	6%	**Comparison between two different instrument model**	
- Medium (M)	4 %	Individual animal milk samples	
- High (H)	3 %	Relative standard deviation of s_{yx} %	8 %
Calibration		Number of individual animal milk samples, Na	100
Mean bias		Herd bulk milk samples'	
Relative mean bias	± 5%	Relative standard deviation of s_{yx} %	8%
Slope,b	1 ± 0,05	Number of herds, Nh1	5
		Number of herd bulk milk samples'	60

6 Phase I - Repeatability

6.1 Repeatability test

The repeatability test is one of the basic and most important tests to perform during the validation study. The repeatability describes the minimum instrument variance results and it is part of intra- and interlaboratory reproducibility.

The data obtained during the stability tests performed in Phase I with pilot samples at three different somatic cell count levels on one instrument were used to calculate the instrument repeatability. Pilot samples with a low, medium and high concentration of somatic cells were prepared using a blank milk sample (semi skimmed UHT milk with 1ml/L polypropylene glycol 2000 and 0,04% bronopol) spiked with a milk leucocyte suspension. Samples from each cell count level were measured in triplicate (n=3) with the Fossomatic™ 7 in random order every 20 min during a working day with 20 checks in total. Routine individual raw cow's milk samples were run in between.

The standard deviation of repeatability (sr), was calculated according to the ICAR protocol and ISO 8196-3|IDF 128-3:2009.

The results reported in table 2 are r_{low} = 11%, r_{medium} = 5%, r_{high} =3% and $r_{overall}$ = 6%.

Table 2. Repeatability (r) on three pilot milks (n=20 for each milk).

				sr	r	sr%	r%	Unit	Limit r %
Repeatability Phase I	r	Low	90	3,6	10,188	4%	11	103 cell/ml	28
		Medium	508	8,1	22,923	2%	5	103 cell/ml	11
		High	1520	14,9	42,167	1%	3	103 cell/ml	5
		Overall					**6**		**11**

The repeatability will be further evaluated with the ILS (see 11.13).

6.2 Conclusion

The instrument's repeatability complies with the ICAR limits in the entire range tested and at the different SCC levels.

7 Phase I - Intralaboratory reproducibility

7.1 Intralaboratory reproducibility test

Intralaboratory reproducibility (Rintra) was checked by analysing pilot samples at three different somatic cell count levels in random order every 20 minutes during a working day with 20 checks in total. Routine individual cow milk samples were run in between.

The standard deviation of repeatability (sr), the standard deviation of means (sx), the standard deviation between checks (sc) and the standard deviation of daily reproducibility (sRdaily) were calculated and are listed in Table 3. ICAR compared the data of standard deviation of daily reproducibility with the limit of standard deviation of intralaboratory reproducibility (sRintra). The reason for this is that the pilot samples have been analysed during the working day (different times on the same instrument) so the operative conditions were intermediate between the repeatability and reproducibility. This situation is described by ISO 13366-2|IDF 148-2 as intra-laboratory reproducibility.

Table 3. Intralaboratory reproducibility (R intra) on three pilot milks (n = 20 for each SCC level).

				s_r	s_x	s_c	s_{Rintra}	R_{intra}	s_{Rintra}	R_{intra}	Limit R_{intra}
				*10³ cells/ml	*10³ cells/ml	*10³ cells/ml	*10³ cells/ml	*10³ cells/ml	%	%	%
Stability Phase I	R_{intra}	Low	90	3,6	5,8	5,4	6,5	18,2	7%	20	**20**
		Medium	510	8,1	17,7	17	18,9	52,92	4%	10	**14**
		High	1520	14,9	29,3	28	31,8	89,04	2%	6	**11**
		Overall								12	**15**

7.2 Conclusion

The instrument's daily reproducibility (Rintra) in phase I complies with the ICAR limit. More specifically, the overall intralaboratory reproducibility $R_{intraoverall}$ = 12% is within the ICAR limit of 15%.

8 Phase I - Carry over

8.1 Carry over test

ICAR protocol, ISO 8196-3|IDF 128-3 and ISO 13366-2|IDF 148-2 define the carry-over as the residual volume of the previous sample as a percentage of the total volume in the instrument flow cell after a single pumping sequence of a sample through the instrument cell.

Internal factors/issues affecting carry over include pump settings, flow system deficiencies and compensation factors. External factors affecting carry-over include transfer from the stirrer and pipette.

After each measurement, the analytical circuit of FM 7 is cleaned to minimise the transfer of a portion of a milk sample to the successive sample.

The carry over tests were executed during the Microval validation process. One preserved blank milk and spiked milk were analysed in the following sequence 20 times:

(blank 1, blank 2, high milk 1, high milk 2)1, (blank 1, blank 2, high milk 1, high milk 2)2…(blank 1, blank 2, high milk 1, high milk 2)20;

The carry over (CO) was calculated from:

$CO = [(\Sigma \text{ (BLANK 1)} - \Sigma \text{ (BLANK 2)}) / (\Sigma \text{ (MILK 2)} - \Sigma \text{ (BLANK 2)})] \times 100$

The obtained value has to be lower than 2% according to ISO 13366-2/IDF 148-2 and ICAR protocol.

For each somatic cell level the ratio CH/L and CL/H have been calculated, see Table 4.

Table 4. Carry-over results for Fossomatic 7.

	Carry Over ratio H/L	Carry Over ratio L/H
High 1 (ca. 500*10³ cells/ml)	0,14	0,48
High 2 (ca. 1000*10³ cells/ml)	0,07	0,14
High 3 (ca. 3000*10³ cells/ml)	0,05	0,32
Limit CO		2%

8.2 Conclusion

The outcome of the carry over tests complied with the ICAR limit of 2% for all the SCC levels tested.

9 Phase I - Linearity

9.1 Linearity test

Linearity expresses the constancy of the ratio between the increase in the concentration of an analyte and the corresponding measurement result. Good linearity is relevant in checking the calibration, for instance when reference materials with a low and a high somatic cell count are used, to prepare the intermediate concentrations.

The ratio of De (= range of residuals) and Dc (= range of concentration) was calculated with:

$$r = \frac{(e_{max} - e_{min})}{(M_{max} - M_{min})} \times 100$$

Where:

e_{max} is the numerical value of the maximum residual from the regression;

e_{min} is the numerical value of the minimum residual from the regression;

M_{max} is the numerical value of the upper measured value for the concerned set of samples;

M_{min} is the numerical value of the lower measured value for the concerned set of samples.

During the Microval validation work, Qlip prepared two sets of samples with a concentration ranging from 0 to 10.000.000 cells/ml. The blank milk was spiked with milk leucocytes suspension to obtain different concentrations of somatic cells. The first set was analysed in increasing concentration of cells, the second set was analysed in decreasing concentration of cells, each with four replicates per sample for a total of eight results per sample.

The calculated ratios r amounted to r range $_{0\text{-}10.000*10^3}$ = 1,80% and r $_{\text{range } 100*10^3\text{-}1.500*10^3}$ = 0,8%.

9.2 Conclusion

Fossomatic ™ 7 showed linear behavior in the routine working range (100.000-1.500.000 cells/ml) and also in the wider range of 0-10.000.000 cells/ml. The instrument complies with the requirements of equal or less than 2% in the ICAR protocol, ISO 8196-3|IDF 128-3 and ISO 13366-2|IDF 148-2.

10 Phase I – Accuracy

The accuracy of Fossomatic ™ 7 was evaluated against Fossomatic ™ FC as anchor method. The accuracy test was performed as follows:

a) Phase I: Fossomatic ™ 7 vs Fossomatic ™ FC (10.1)

b) Calibration data (11.8)

c) Comparison of the instrument measurement results with calibration sample values (11.9)

10.1 Accuracy test Fossomatic ™ 7 vs Fossomatic ™ FC

Qlip selected 220 single cow milk samples in a range from 50.000-2.000.000 cells/ml and 179 herd bulk cow milk samples. Herd bulk cow milk samples with a concentration higher than 500*103 cell/ml were prepared through spiking with a milk leucocyte suspension. These samples were analysed in parallel with the two instruments in two replicates per sample. Both instruments were set with slope=1 and bias=0. Before calculation of the standard deviation of accuracy, the repeatability was checked. All results obtained complied with the ICAR limits, see Table 5.

Table 5. Repeatability on single cow milk samples and herd bulk cow milk samples.

				Single cow milk sample	Herd bulk cow milk samples	Unit	
				r%	r%	103 cell/ml	Limit r %
Repeatability Phase I (data from the accuracy test)	r	Level 1	50-200	7	9	103 cell/ml	17
		Level 2	201-400	6	6	103 cell/ml	14
		Level 3	401-650	6	5	103 cell/ml	11
		Level 4	650-1000	4	4	103 cell/ml	8
		Level 5	1000-1500	4	2	103 cell/ml	8
			Overall	5,4	5,2		11

Based on the mean result of each of the two replicates the standard deviation of accuracy was calculated for the two groups of samples. The results obtained for the standard deviation of accuracy were $s_{yx\_singlecow}$ % = 5,8% and s_{yx_herd} = 4,1 % (Table 6).

Table 6. Accuracy Fossomatic ™ 7 versus Fossomatic ™ FC.

	Individual cow milk samples	Herd bulk cow milk samples	Limit
n	220	179	
range SCC*10^3 /ml	50-2000	50-2000	
s_{yx} %	5,8	4,1	8

10.2 Conclusion

The accuracy test executed, comparing Fossomatic™ 7 with Fossomatic™ FC, resulted in a standard deviation of accuracy syx below the proposed limit of 8 %.

11 Phase II - Interlaboratory study

An ILS was organised in order to validate some of the criteria examined in phase I in one single laboratory and to calculate the precisions, repatability and reproducibility, of Fossomatic™ 7 instruments situated in different laboratories .

Qlip organised the ILS according to ISO 5725-1, ISO 5725-2 and IDF Bulletin 453/2012. It shall be noted that Qlip is ISO 17043 accredited and the data analysis of the ILS is within the scope of this accreditation.

11.1 Participant laboratories

Eight laboratories from eight countries participated in the validation study with a total number of 10 Fossomatic ™ 7 instruments. The names of the laboratories are presented in alphabetic order in Table 7.

Table 7. Participant laboratories in the ILS.

Laboratory Name	Country
Cooprinsem	Chile
Dairygold Co-Operative	Ireland
Eastern Laboratory Services	USA
Federazione Latterie Alto Adige Soc. Agr. Coop.	Italy
LIGAL	Spain
Milchprüfring Bayern	Germany
National Milk Laboratories	UK
Qlip	Netherlands

11.2 ILS design

The participating laboratories were provided with the following material (per instrument):

- 1 vial of 60 ml containing water for temperature control upon arrival (code: water)
- 2 vials of 60 ml containing Fossomatic ™ rinse sheath solution, (code: blank)
- 2 vials of 60 ml containing UHT semi skimmed milk, (code: blank milk)
- 1 vial Fossomatic™ Adjustment Sample (code: FMA)
- Eight single cow milk samples, 2 bulk milk samples and 2 UHT semi skimmed milk samples were split in double blind each. In this way a set of, in total, twenty-four vials was created (24 vials of 60 ml each containing preserved raw milk for measurements with Fossomatic™ 7 (e.g sample 1 was split in vials FM 4 to FM 10, see Table 8).

Note: samples were preserved with 0,04% bronopol (end concentration in the milk).

- 5 vials with QSE calibration samples, lyophilized, (code SCC 1 to SCC 5). QSE calibration material is accredited DAkkS number D-RM-20961-01-00 for DIN EN ISO 17034:2017. The material was characterized by considering data obtained with the microscopy method according to ISO 13366-1 | IDF 148-1 and the luoroptoelectronic method operated according to ISO 13366-2 | IDF 148-2.

Table 8. Sample identification and type of milk.

Milk	Sample ID	Vial ID	
Individual cow Milk	1	FM 4	FM 10
	2	FM 16	FM 22
	3	FM 6	FM 19
	4	FM 1	FM 7
	5	FM 9	FM 21
	6	FM 12	FM 18
	7	FM 3	FM 5
	8	FM 15	FM 17
Herd mIlk	9	FM 11	FM 13
	10	FM 23	FM 24
UHT Semi Skimmed Milk	11	FM 2	FM 8
	12	FM 14	FM 20

11.3 Transport conditions and delivery time

All samples were sent on 25 of November and delivered between 26 November and 2 December 2019. The samples retained in the custom office were kept refrigerated. The samples temperature at arrival was between 1°C and 6,9°C. The sample quality reported by all the laboratories was good and all the samples delivered have been analysed. The two laboratories that received the parcel on 30 November and on 2 December did not report any problem regarding the sample quality

11.4 Sample homogeneity

Each of the 12 FM samples was tested in two replicates. The sample homogeneity was checked through the fat concentration. According to ISO 13528 the variance between vials (se) should not be bigger than 0,3*sR of the method used to check the homogeneity. Table 8 lists the results obtained and the calculated limit for each level of concentration. All the samples tested showed a standard deviation between vials (se) that was lower than the calculated limit.

Table 9. Sample homogeneity between vials.

*10^3 Cells/ml

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
mean value	172	131	294	372	515	1023	1263	1352	228	273
s_r	3,742	5,79	5,48	16,34	4,60	17,79	4,135	14,54	7,25	9,35
r	10,77	10,47	15,34	45,75	12,88	49,81	4,37	40,71	20,30	26,18
s_r%	2,2	2,9	1,9	4,4	0,9	1,7	0,1	1,1	3,2	3,4
s_R ISO 13366-2	15,2	12,0	23,6	28,0	34,9	57,7	67,2	70,3	19,3	22,3
se	2,57	2,35	3,69	0,00	6,37	10,24	20,01	0,59	0,00	1,27
se%	1,5	1,8	1,3	0,0	1,2	1,0	1,6	0,0	0,0	0,5
Limit se	4,57	3,59	7,09	8,39	10,48	17,30	20,15	21,08	5,80	6,70
Limit se%	2,7	2,7	2,4	2,3	2,0	1,7	1,6	1,6	2,5	2,5

11.5 ILS execution

All the participating laboratories received detailed instructions on how to treat the samples and how to prepare the instrument according to specific manufactures instructions in order to obtain the best standardised conditions. All the tests were executed on 29 November 2019 with the exception of one set of samples analysed on 28 November and two sets on 2 December. It was requested by ICAR to set slope=1 and bias=0 on all the instruments before analysing the ILS samples.

As initial step, it was requested to analyse the blank and the blank milk samples in twelve replicates each. The mean results should not exceed 3.000 cells/ml and all the individual results should be below 8.000 cells/ml - if not, the procedure had to be repeated. FMA samples were analysed next. Thereafter, the ILS samples (ID Vials FM1-FM24) were tested in two replicates. Successively, the laboratories reconstituted the lyophilized milk provided (QSE calibration material) and analysed each calibration material in five replicates.

11.6 Laboratory results and statistical treatment

The results of total somatic cells were statistically processed as follows:

a) Evaluation of instrument checks as blank, blank milk and FMA

b) Calibration data (11.8)

c) Comparison of the instrument measurement results with calibration sample values (11.10)

d) Calculation of precision (11.12)

11.7 Evaluation of the instrument checks

The results are reported in Table 10.

Table 10. Results of quality controls.

1000*cells/ml

Laboratory	Blank Mean	Blank milk Mean	FMA
1 FM7	0	10	411
2 FM7	0	9	414
5 FM7	1	10	391
10 FM7	0	10	398
13 FM7	1	10	413
14 FM7	0	11	419
15 FM7	0	7	449
16 FM7	0	8	399
17 FM7	1	10	442
19 FM7	0	11	404

All the results complied with the indicated limits.

11.8 Calibration data

During the ILS, the ILS samples were analysed using ten different instruments with slope=1 and bias=0. The laboratories analysed five calibration samples in five replicates, from which the mean bias and slope were calculated considering the calibration material value (y-axis) and the instrument results (x-axis). Furthermore, the confidence interval for slope was calculated for each instrument.

Table 11. Mean bias of the instrument measurement results with calibration sample values

	SCC 1	SCC 2	SCC 3	SCC 4	SCC 5	Mean Bias %
Laboratory			**% Bias**			
1 FM7	2	5	7	6	8	6
2 FM7	8	4	5	4	4	5
5 FM7	12	4	1	0	1	4
10 FM7	6	2	4	5	2	4
13 FM7	9	7	7	10	10	9
14 FM7	12	3	2	6	7	6
15 FM7	14	15	16	15	17	15
16 FM7	15	17	17	14	14	15
17 FM7	7	6	5	9	7	7
19 FM7	9	3	4	6	8	6

Table 12. Instrument slope value with the calibration material

	SCC 1	SCC 2	SCC 3	SCC 4	SCC 5	slope	Lowest 95%	Highest 95%
1 FM7	141	280	438	792	1144	0,92	0,90	0,94
2 FM7	149	276	429	775	1103	0,96	0,95	0,97
5 FM7	154	276	405	747	1067	1,00	0,96	1,04
10 FM7	146	273	425	780	1082	0,97	0,94	1,01
13 FM7	151	285	439	821	1159	0,91	0,88	0,93
14 FM7	155	274	419	787	1133	0,93	0,89	0,98
15 FM7	119	225	346	630	882	1,20	1,18	1,22
16 FM7	118	221	341	638	914	1,15	1,12	1,18
17 FM7	148	281	432	808	1128	0,93	0,90	0,96
19 FM7	150	274	427	785	1140	0,93	0,89	0,96

11.9 Conclusion

All the calculated slopes are within the confidence interval. As the instrument accuracy was already evaluated in phase I, with the instrument set with slope=1 and Bias=0, through comparison with

Fossomatic™ FC, the results of this ILS have not been recalculated. The slope evaluation is reported only as informative for the laboratories that participated in the ILS study.

11.10 Comparison of the instrument measurement results with calibration sample values

The difference between the calibration material and measured value with its uncertainty were calculated and reported as informative.

The comparison of $\Delta m \leq U\Delta$ where:

Δm = the absolute difference between the mean measured value and calibration sample value

$U\Delta$ = the expanded uncertainty of Δm

$U\Delta = 2 * u\Delta$ with K=2 with a confidence level of 95%

$u\Delta$ = combined uncertainty of Δm

$u\Delta = \sqrt{u^2_m + u^2_{SRM}}$

u_m = combined uncertainty of measurement result

u_{SRM} =combined uncertainty of the calibration material

u_m was considered the standard deviation of interlaboratory study (s_RILS) obtained for each level of concentration $u_m = s_R$ILS

Obtained results are listed in Table 13.

Table 13. Comparison between instrument results and calibration sample values ($\Delta m \leq U\Delta$).

						Absolute difference Inst.- SRM				
Laboratory	**SCC 1**	**SCC 2**	**SCC 3**	**SCC 4**	**SCC 5**	**SCC 1**	**SCC 2**	**SCC 3**	**SCC 4**	**SCC 5**
1FC7	141	280	438	792	1144	3	14	28	48	87
2FC7	149	276	429	775	1103	11	10	19	31	46
5FC7	154	276	405	747	1067	16	10	5	3	10
10FC7	146	273	425	780	1082	8	7	15	36	25
13FC7	151	285	439	821	1159	13	19	29	77	102
14FC7	155	274	419	787	1133	17	8	9	43	76
15FC7	119	225	346	630	882	19	41	64	114	175
16FC7	118	221	341	638	914	20	45	69	106	143
17FC7	148	281	432	808	1128	10	15	22	64	71
19FC7	150	274	427	785	1140	12	8	17	41	83
SRM Value	**138**	**266**	**410**	**744**	**1057**					
U SRM	**7,3**	**8,2**	**14,8**	**18**	**23,8**					
u SRM	3,65	4,1	7,4	9	11,9					
u SRM /SqRout(5)	1,63	1,83	3,31	4,02	5,32					
sR ISO 13366-2	12,42	22,00	30,00	45,00	59,00					
Bias Limit						25,1	44,2	60,4	90,4	118,5

SRM= Calibration material

11.11 Conclusion

The comparison of the instrument measurement results with calibration sample values indicated that instrument 15 and 16 are out of the limit for 3 or 4 calibration samples and should be calibrated. Because the reproducibility evaluation was done on the raw data obtained with slope =1 and bias= 0 these results are reported only as informative.

11.12 Precision calculation

The precision calculation, specifically repeatability and interlaboratory reproducibility, was done according to ISO 5725-2.

In Table 14 the results of the first replicate of each vial and the first replicate of its double blind sample obtained with slope=1 and bias=0 are reported for each laboratory. These data have been to calculate the instrument precisions, repeatability and reproducibility.

Table 14. Participants laboratory results.

Sample ID	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
Vial ID	FM4	FM10	FM16	FM22	FM6	FM19	FM1	FM7	FM9	FM21
Laboratory										
1 FM7	182	172	129	132	309	306	371	378	506	426
2 FM7	181	178	135	145	309	304	371	376	541	533
5 FM7	185	176	143	132	318	319	388	372	521	534
10 FM7	189	177	142	130	323	308	378	386	544	532
13 FM7	181	183	137	130	310	319	375	379	526	519
14 FM7	181	175	139	138	311	319	372	376	531	525
15 FM7	172	178	138	136	319	311	396	400	539	551
16 FM7	180	175	135	135	313	302	374	393	539	528
17 FM7	160	157	127	119	274	270	346	348	477	476
19 FM7	175	172	126	122	298	302	375	376	491	505

Sample ID	Sample 6		Sample 7		Sample 8		Sample 9		Sample 10	
Vial ID	FM12	FM18	FM3	FM5	FM15	FM17	FM11	FM13	FM23	FM24
Laboratory										
1 FM7	1037	1030	1291	1313	1406	1418	233	220	278	278
2 FM7	1047	1041	1311	1319	1427	1400	226	232	277	281
5 FM7	1064	1067	1336	1312	1493	1456	230	236	295	273
10 FM7	1068	1056	1335	1355	1467	1466	224	226	294	275
13 FM7	1042	1049	1278	1315	1456	1452	237	237	283	270
14 FM7	1033	1084	1333	1314	1436	1425	224	226	281	283
15 FM7	1060	1079	1371	1347	1478	1500	226	233	283	288
16 FM7	1045	1047	1341	1350	1448	1449	234	239	280	281
17 FM7	897	901	1168	1158	1397	1408	220	218	251	248
19 FM7	1015	1013	1284	1281	1386	1394	226	224	271	273

Cochran outlier
** Grubbs oulier
* Grubbs straggler

The statistical analyses identified for laboratory 1 FM7 sample 5 and for laboratory 14 FM 7 sample 6 as Cochran outliers. These outliers are not associated to the delayed delivery. These data were omitted in further calculations. The identified straggler data was retained in the statistical elaboration.

The calculated precision values per sample and overall are reported in Table 15.

Tab 15. Repeatability (r) and Reproducibility (R)..

		ILS result				Interpolated limits			
		r		R		r, limit ISO 13366-2 IDF 148-2		R, limit ISO 13366-2 IDF 148-2	
Sample	Mean Instruments result *10³cells/ml	*10³ cells/ml	%	*10³ cells/ml	%	*10³ cells/ml	%	*10³ cells/ml	%
Sample 2	134	14	11	14	11	23	17	34	25
Sample 1	176	13	8	22	12	29	16	44	25
Sample 9	229	11	5	18	8	35	15	54	24
Sample 10	277	21	7	33	12	40	14	63	23
Sample 3	307	16	5	42	14	16	5	68	22
Sample 4	377	18	5	38	10	47	12	79	21
Sample 5	523	20	4	63	12	55	11	99	19
Sample 6	1031	17	2	147	14	86	8	162	16
Sample 7	1306	40	3	158	12	110	8	192	15
Sample 8	1438	35	2	97	7	121	8	205	14
Overall			5		11		12		20
ICAR Limit							11		

11.13 Conclusion repeatability

The repeatability with samples with the same level of concentration and the overall repeatability is favourable to interpolated limit values from ISO 13366-2|IDF 148-2 for all samples tested. Furthermore, the overall repeatability $r_{overall}$ = 5% is smaller than the indicated ICAR limit of r= 11%.

The repeatability value means that the absolute difference between two independent single test results (R) obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should in not more than 5 % of cases be greater than 5% for the instrument Fossomatic™ 7.

11.14 Conclusion reproducibility

The results show that the overall instrument reproducibility values are smaller than the interpolated limit values from ISO 13366-2|IDF 148-2 for all samples tested. Furthermore the overall reproducibility $R_{overall}$ = 11% is smaller than the indicated ISO/IDF limit of R_{Limit}=20 %.

The reproducibility value means that the absolute difference between two independent single test results (R) obtained using the same method on identical test material in different laboratories by different operators using the same equipment within a short interval of time, should in not more than 5 % of cases be greater than 11% for the Fossomatic™ 7.

12 Conclusion

The data obtained during phase I and phase II (ILS) provided robust evidence of an adequate instrument performance. The instrument performance obtained in phase I was confirmed in phase II. Considering the range tested during the ILS (130.000-1.500.000 cells/ml), the FM 7 precision is:

Repeatability (R) = 5%

Reproducibility (R) = 11%

The values for each level of SCC concentration tested are reported in Table 15.

For this instrument, a specific robustness test has not been performed but this will be done in the coming years and provided in connection with the certification renewal. However, the robustness test is not mandatory for the ICAR certification..

The instrument complies with all limits defined in Procedure 1 of Section 12 of the ICAR Guidelines – Protocols for Evaluation of Milk Analyses for ICAR Approval, aligned with ISO 8196-3|IDF 128-3:2009

– Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis for all the criteria tested and ISO 13366-2|IDF 148-2.

ICAR certifies the performance of Fossomatic ™ 7 based on above described studies and results thereof. All studies were executed by independent and accredited laboratory, Qlip. In addition an extensive international validation study was performed under accreditation

13 Acknowledgment

ICAR thanks QLIP (NL), all the laboratories that participated in the interlaboratory study for the qualified work done and last but not least the members of ICAR Milk Analysis Sub Committee for the report revision and approval.

14 Reference documents

- Protocol for the Evaluation of Milk Analysers for ICAR Approval: 2019. https://www.icar.org/index.php/icar-recording-guidelines/
- ISO 8196-3|IDF 128-3 :2009 – Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis.
- EN ISO 13366-2|IDF 148-2:2006 Milk - Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-optoelectronic counters.
- T.P.J. Linsinger, ERM Application note 1: Comparison of a measurement result with the certified value, https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes (last accessed on 16 January 2020)
- ISO 17034:2016 General requirements for the competence of reference material producers
- Bulletin of the IDF 453/ 2012: Guidance for the evaluation of precision characteristics of physicochemical quantitative analytical methods for milk and milk products
- ISO 5725-1:1994 Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions
- ISO 5725-2:2019 Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method