ICAR RE-CERTIFICATION

Company name: Foss Analytical A/S

Instrument name: Fossomatic™ 7 DC

Milk species: Cow milk

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Approved and revised by Milk Analyses Sub Committee

Version March 2025

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1.Preface

Fossomatic™ 7DC was fully certified by ICAR in May 2020 with the organization of a interlaboratory study.

The purpose of this report is to evaluate if the instrument, introduced on the market at the end of 2016 is still meeting the requirements of the previous granted certification after five years.

The instrument is applied in central milk testing laboratories and dairy laboratories for milk recording analysis and milk payment testing.

The data obtained in the ICAR proficiency test and from the interviewed laboratories have been used for the evaluation.

The limits reported in reference documents reported below and the above results obtained, will be considered in the evaluation.

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
- ISO 13366-2|IDF 148-2 Milk — Enumeration of somatic cells — Part 2: Guidance on the operation of fluoro-opto-electronic counters

2.Principle

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

3.Scope

The scope of the re-certification is total somatic cell counting in raw cow milk.

4.Data evaluated

- 8 rounds of ICAR Proficiency test from March 2021 to March 2025
- Raw data and report from ARAL Crema (ITALY) 2024 on 3 Fossomatic™ 7 DC
- Report from Lactanet on the Fossomatic™ 7 DC stability and robustness

5.ICAR proficiency tests

The ICAR proficiency test data from March 2021 to March 2025 have been evaluated. For the purpose of re-certification, these data were used to calculate and assess the repeatability of the Fossomatic™ 7DC. The reproducibility has been not evaluated because is influenced by the calibration settings specific to each laboratory.

For the total somatic cell the repeatability of the Fossomatic™ 7DC was found to be:

- r = 40,28 cell*1000/ml
- sr% = 2,4

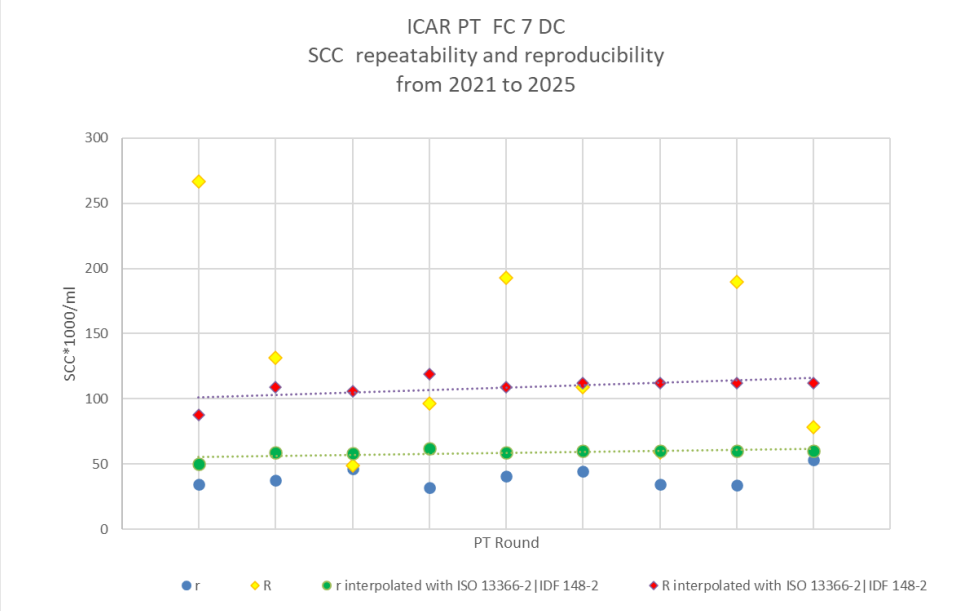
These values are below the limit defined by ISO 8196-3 | IDF 128-3, which is sr % ≤ = 4% for the whole range

Table 1. and Figure 1. report the results of each proficiency test for Fossomatic™ 7 DC and its overall performance.

Table 1. ICAR PT Total Somatic cell Counting (SCC) - Summary Table

	rt0321	rt0921	rt0322	rt0922	rt0323	rt0923	rt0324	rt0924	rt0325	Overall
count	11	4	5	7	10	4	7	6	9	
n samples	110	40	50	70	100	40	70	60	90	630
n.outliers Cochran	4	0	1	1	1	0	0	0	1	
n outliers Grubbs	1	0	0	1	0	0	0	0	7	
average	494	591	547	668	608	603	612	618	616	595
s_r^2	152,912	178,6375	274,6875	129,1357	212,0961	253,5	151,7357	148,3	361,9055	206,99
s_r	12,37	13,37	16,57	11,36	14,56	15,92	12,32	12,18	19,02	14,39
s_L^2	8918,621	2036,946	34,0525	1050,2	4545,798	1257,096	286,3774	4447,278	424,8227	
s_R^2	9071,533	2215,583	308,74	1179,335	4757,894	1510,596	438,1131	4595,578	786,7282	2762,678
s_R	95,24	47,07	17,57	34,34	68,98	38,87	20,93	67,79	28,05	52,56
s_r_relative	2,5	2,3	3,0	1,7	2,4	2,6	2,0	2,0	3,1	2,4
sR_relative	19,3	8,0	3,2	5,1	11,3	6,4	3,4	11,0	4,6	8,8
repeatability	34,62	37,42	46,41	31,82	40,78	44,58	34,49	34,10	53,27	40,28
Reproducibility	266,68	131,80	49,20	96,16	193,14	108,83	58,61	189,81	78,54	147,17
repeatability_relative	7,0	6,3	8,5	4,8	6,7	7,4	5,6	5,5	8,6	6,7
Reproducibility_relative	53,9	22,3	9,0	14,4	31,7	18,0	9,6	30,7	12,7	22,5

Figure 1. ICAR PT Total Somatic cell Counting (SCC)



6.Stability

To assess instrument stability, Aral Crema (IT) provided data from pilot samples analyzed throughout 2024 using three Fossomatic™ 7 DC instruments. A total of 34,556 pilot samples were tested, of which 2.085 exceeded the specified tolerance limits for somatic cell count, resulting in a deviation rate of 6%.

Pilot samples are analyzed systematically. If a sample falls outside the tolerance limits, a second pilot sample is tested. If this second sample also falls outside the limits, the analysis is automatically halted. At that point, the device operator must identify and resolve the root cause of the deviation. Following corrective action, two new pilot samples must be analyzed during system start-up. Additionally, the most recent sample that previously met specifications, along with subsequent samples, must be reanalyzed to confirm instrument stability.

To evaluate intralaboratory reproducibility, pilot sample results from one working day per month throughout 2024 were analyzed. In the selected working days all the 3 Fossomatic™ 7 DC were operative.

A total of 1.558 pilot samples, drawn from the non-compliant cases, were included in the statistical evaluation.

These results were subjected to statistical analysis.
The standard deviation of intralaboratory reproducibility (sRintra) was calculated considering the pilot repeatability (sr) and the pilot standard deviation between instruments (sL) of 12 working routine day of 2024.

Table 2. shows the intra laboratory relative repeatability and intra-laboratory reproducibility calculated for each working session on the 3 instruments.

Both criteria are in the limit reported in the ISO 8196-3|IDF 128-3 for a low range of concentration.

sr % FM 7 DC=6,0 % is in the limit of sr %_ ≤ 6%

sRintra % FM 7 DC=6 % is in the limit of sRintra %_ ≤ 7%

Table 2. Results of pilot sample on 3 instruments

		02/01/2024	01/02/2024	01/03/2024	02/04/2024	02/05/2024	03/06/2024	02/07/2024	01/08/2024	06/09/2024	01/10/2024	04/11/2024	04/12/2024	Overall	Limit ISO 8196-3 |IDF 128-3
SCC*1000/ml	Mean	153	176	154	223	143	282	331	280	264	184	232	200	218	
	s	5,53	8,10	5,82	7,55	6,14	8,86	10,09	9,01	9,12	6,86	9,69	7,44	7,988	
	s%	3,62	4,59	3,77	3,39	4,30	3,14	3,05	3,22	3,45	3,73	4,18	3,71	3,66	
	sr	9,10	10,30	9,20	12,30	8,60	14,40	15,90	14,40	13,80	10,60	12,70	11,30	12,104	
	SL_{intra}	0,000	3,551	0,000	0,000	0,874	0,000	0,000	0,000	0,000	0,000	3,629	0,000	1,487	
	SR_{intra}	9,100	10,895	9,200	12,300	8,644	14,400	15,900	14,400	13,800	10,600	13,208	11,300	12,195	
	R_{intra}	25,480	30,506	25,760	34,440	24,204	40,320	44,520	40,320	38,640	29,680	36,983	31,640	34,146	
	sr%	6	6	6	6	6	5	5	5	5	6	5	6	6	6%
	SR_{intra} %	6	6	6	6	6	5	5	5	5	6	6	6	6	7%
	n	25	154	176	122	133	131	135	125	146	151	138	122	1558	

7.Robustness

7.1 Report ARAL Crema

ARAL Crema reported "*the 3 instruments were installed between 2018 -2021.*
The equipments presents various issues: they are "delicate" instruments that require constant minor maintenance (e.g., replacing tubes, cleaning sensors). Specifically, we experience problems with the incubator, which contains 12 very small valves. The new version of the incubator has certainly brought some

improvements, although certain issues persist. The RSH unit consistently has sensor issues (we even found rust near the sensors). The measurement cell gets dirty much more easily than the one installed on the FC models, resulting in significant time lost for cleaning and subsequent focusing.
The daily use of FMA liquid to check the focus has certainly led to increased costs. FMA is also used to perform focusing after cleaning the cell.
We have a maintenance contract for the four installed instruments that includes two visits per year. In 2024, one of the four devices required an additional two maintenance interventions by a FOSS technician (in addition to the ones included in the contract).
Another issue concerns the output of analysis data: after every interruption, the operator must wait several minutes for the instrument to go into standby mode (due to the incubator's timing). It's clear that when the instrument has problems and the operator is forced to stop the analysis, this negatively impacts the routine resulting in 500 samples/hour analyzed.
At ARAL, we have trained operators who are capable of performing maintenance on the Fossomatic™ 7 DC, but they often need phone support from FOSS technicians due to difficulties in resolving the recurring instruments issues."

7.2 Report ICAR Milk Analyses members

The Fossomatic™ 7 DC requires more frequent maintenance visits due to its delicate design and heightened sensitivity. As a result of recurring interruptions and interventions, the instrument typically processes samples at an average rate of 300 per hour under routine conditions.. It was observed an average non-compliance rate of 3% for the pilot samples. The primary maintenance challenges arise from the instrument's high sensitivity to internal noise. Frequent alerts include "pinch valve malfunction" and "baseline error," both commonly linked to internal vibration or noise. These issues are often resolved by replacing vibration dampers or addressing exposed tubing within the unit. Another critical component prone to maintenance is the incubator, which tends to require more frequent repairs. Overall, the increased complexity of the Fossomatic™ 7 DC—featuring more sensitive components such as additional pinch valves, the buffer/dye mixing chamber, and a greater susceptibility to mechanical vibrations—contributes significantly to its higher maintenance demands compared to the Fossomatic™ 7.

8.Foss report on the software change log

- The following information are about new or changed functionality compared to earlier versions of the Foss Integrator Software and applied to Fossomatic™ 7 and Fossomatic™ 7 DC

8.1 Version 3.6.3 Release March 2025

- Using prediction input display can cause internal error on Fossomatic 7. If the user would inspect samples selecting them in the prediction input display to see their PHA curves, Foss Integrator would crash.

8.2 Version 3.6.2 Release July 2024

- A customer lost several samples as the software did not react properly to an internal crash, instead it kept running. Fault handling was improved to prevent such situations going forward.
- Connection loss fixed.
- FMA lot settings in Spanish adjusted.

8.3 Version 3.6.1 Release July 2023

- Support for SQL 2014 and Win 7 ended

8.4 Version 3.6.0 Release October 2022

- After running the rinse job, Foss Integrator asks to put a cup under the pipette and do the job a second time. In one of the places there were errors after the job was done. This has been fixed.

8.5 Version 3.5.0 Release November 2021

- Extra suction was implemented to avoid pipette drip when instrument change mode from Stop to Standby and Standby to Stop.
- Introduced a pinch valve startup exercise. This is implemented to avoid low cell count in first sample after going from Standby to measure mode, which can be caused by slow/dozy pinch valves.
- A notification to inform customers that intercept adjustment should not be performed when doing S/I adjustment on a Fossomatic. This will assist customers getting correct and understandable results - especially on a Fossomatic 7 DC where the DSCC value is disabled or set to zero when cell count is below 50k. The new notification is applied to all Fossomatic instruments: Fossomatic 7 DC, Fossomatic 7 and Fossomatic FC.

8.6 Version 3.3.0 Release December 2020

- The warning and Error descriptions and their hints were improved to be more informative.
- The following information are about new or changed functionality compared to earlier versions of the Foss Integrator Software.

9.Conclusion

The results show that the Fossomatic™ 7 DC repeatability and overall intra laboratory reproducibility for total somatic cell counting are lower than limits reported in ISO 8196-3|IDF 128-3.
The data provided by Aral Crema comments received from Milk analyses SC, provided the same evidence of the instrument's sensibility reported in the ICAR certification in 2020.

ICAR report 2020 "*The overall conclusion is that the FM 7 DC is an instrument that requires somewhat more attention and is somewhat more sensitive to the quality of milk samples compared to Fossomatic™ 7. This is clearly explainable by the fact that new and different technology and dye solution are used compared to Fossomatic™ 7. The sample quality is critical for obtaining reliable results".*