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MICROVAL VALIDATION REPORT

Comparative Study

MicroVal Project 2016LR63

Validation of Fossomatic 7 DC™ (FOSS) for Enumeration of Somatic Cells in Raw Cow's Milk against EURL MMP criteria

Confidential

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Summary

This MicroVal validation report presents the results obtained with the newly developed Fossomatic™ 7 DC instrument (FOSS Analytical A/S) for enumeration of somatic cells in raw cow milk. The instrument was validated against the criteria in the document of European Union Reference Laboratory for Milk and Milk Products (EURL MMP) from January 2013 (1), which refers to performance criteria in ISO 8196-3 (2) and ISO 13366-2 (3) and compared with the already approved Fossomatic™ FC (MicroVal certificate 2015LR55).

Conclusions of the method comparison study

Fossomatic™ 7 DC performance characteristics determined according to ISO 8196-3 and ISO 13366-2 are:

- Fossomatic™ 7 DC functions stable through the working day
- Repeatability (*r*) per cell count level:
  - Low (ca. 181.10^3 cells/mL) 11 % *(ISO 13366-2: ≤ 17 %)*
  - Medium (ca. 563.10^3 cells/mL) 6 % *(ISO 13366-2: ≤ 11 %)*
  - High (ca. 1 583.10^3 cells/mL) 3 % *(ISO 13366-2: ≤ 9 %)*
- Carry-over per cell count level (*ISO 13366-2: for each cell count level CO < 2 %)*
  - Low (ca. 500.10^3 cells/mL) $C_{H/L}$ = 1,49 %; $C_{L/H}$ = 0,00 %
  - Medium (ca. 1 000.10^3 cells/mL) $C_{H/L}$ = 0,05 %; $C_{L/H}$ = 0,12 %
  - High (ca. 3 000.10^3 cells/mL) $C_{H/L}$ = 1,44 %; $C_{L/H}$ = 0,64 %
- Linearity (r_C): 1,7 % *(ISO 13366-2: r_C ≤ 2 %)*
- Lower limit of quantification (L_Q): 16.10^3 cells/mL
- Upper limit of quantification: 10 000.10^3 cells/mL
- High fat (up to 8 %) and protein (up to 5,5 %) content of the milk do not relevantly influence the somatic cell count results with the Fossomatic™ 7 DC.

Conclusions of the comparison of Fossomatic™ 7 DC and Fossomatic™ FC

The results obtained from the comparison of Fossomatic™ 7 DC and Fossomatic™ FC are:

- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level using bronopol-preserved individual raw cow milk:
  - Cell level 50-200.10^3 cells/mL 15 % *(ISO 13366-2: ≤ 20 %)*
  - Cell level 201-400.10^3 cells/mL 11 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401-600.10^3 cells/mL 9 % *(ISO 13366-2: ≤ 14 %)*
  - Cell level 601-1 000.10^3 cells/mL 9 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 1 000-1 500.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 11 %)*
- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in:

Unpreserved raw herd bulk cow milk

- Cell level 50-200.10^3 cells/mL 19 % *(ISO 13366-2: ≤ 20 %)*
- Cell level 201-400.10^3 cells/mL 16 % *(ISO 13366-2: ≤ 17 %)*
- Cell level 401-600.10^3 cells/mL 13 % *(ISO 13366-2: ≤ 14 %)*

Unpreserved raw herd bulk cow's milk spiked with milk leucocyte suspension

- Cell level 601-1 000.10^3 cells/mL 20 % *(ISO 13366-2: ≤ 11 %)*
- Cell level 1 000-1 500.10^3 cells/mL 21 % *(ISO 13366-2: ≤ 11 %)*

- Standard error (s_{yx}) with natural log transformed results was:
  - for bronopol-preserved individual raw cow's milk, s_{yx} = 0,06 Ln.10^3 cells/mL
  - for unpreserved raw herd bulk cow's milk, s_{yx} = 0,05 Ln.10^3 cells/mL
- A small significant, but irrelevant, deviation of the regression line from the identity function was observed. Close correlation was demonstrated between the results obtained with both instruments on unpreserved and bronopol-preserved raw milk samples
- Results obtained with Fossomatic™ 7 DC and Fossomatic™ FC are equivalent for all cell count levels when applied on unpreserved and bronopol-preserved cow's milk samples. The use of sodium azide as a preservative can effect the equivalence of the results obtained with both models (e.g., high correlation but inter-laboratory reproducibility slightly beyond ISO requirements).

Final conclusion methods' comparison study

The final conclusion of the validation study is:

The Method Comparison Study of Fossomatic™ 7 DC (FOSS Analytical A/S) and the direct comparison with Fossomatic™ FC (MicroVal certificate 2015LR55) show that the results obtained with both instruments are equivalent using unpreserved and bronopol-preserved cow's milk samples. All results of the tests performed in this study confirm that the new method complies with the criteria of the EURL MMP document.

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1. Introduction

The Fossomatic™ 7 DC flow cytometer from FOSS Analytical A/S is a dedicated instrument for high-throughput enumeration of somatic cells and, additionally, determination of differential somatic cell count in raw milk.

Since independent validation is a critical success factor for the acceptance of the Fossomatic™ 7 DC as an instrumental method for the enumeration of somatic cells in raw milk in light of EU Regulation No 2074/2005, modified by EU Regulation No 1664/2006, the Fossomatic™ 7 DC has to be validated against the European criteria published in an EURL MMP document from January 2013 (1). The EURL MMP document for validation of alternative methods refers to performance criteria in ISO 8196-3 (2) and ISO 13366-2 (3).

Fossomatic™ 7 DC is a new generation of Fossomatic instruments for somatic cell counting in raw milk. FOSS launched Fossomatic™ 7 in October 2016 and Fossomatic™ 7 DC in June 2017. While Fossomatic™ 7 is for enumeration of somatic cells only, Fossomatic™ 7 DC allows simultaneous determination of somatic cell count (SCC) and differential somatic cell count (DSCC). Differential somatic cell count is a new parameter providing more detailed information on the udder health status of dairy cows and its main application is seen on individual cow milk samples (i.e., dairy herd improvement) (4). The Fossomatic™ 7 and Fossomatic™ FC are already granted with MicroVal certificates. The hardware and measuring principle of the different models is highly similar, however the Fossomatic™ 7 DC has some differences (e.g., using acridine orange as a fluorescent dye instead of ethidium bromide). Furthermore, the determination of SCC is done using an algorithm based on dot-plots on Fossomatic™ 7 DC (5) instead of pulse height amplitude (PHA) diagrams as on Fossomatic™ 7 or FC. Evaluation of the performance of the Fossomatic™ 7 DC in terms of DSCC was not subject of this study.

The performance characteristics of the Fossomatic™ 7 DC with total somatic cell count are demonstrated during the methods' comparison study for the matrix raw cow's milk. Its accuracy is demonstrated by comparison with results obtained with the Fossomatic™ FC.

This MicroVal validation report presents the results of an executed methods' comparison study as prescribed in the EURL MMP document from January 2013 and results of comparison of two Fossomatic models.

1.1. Principle of the alternative method

The Fossomatic™ 7 DC 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 - acridine orange - 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 must ensure that single cells are separately counted.

Between analysis of subsequent samples the flow system is thoroughly cleaned to reduce the carry-over to a minimum as well as the risk of build-up and clogging inside the analyser.

1.2. Scope

Raw cow's milk

1.3. Restriction of use

None

1.4. Reference method

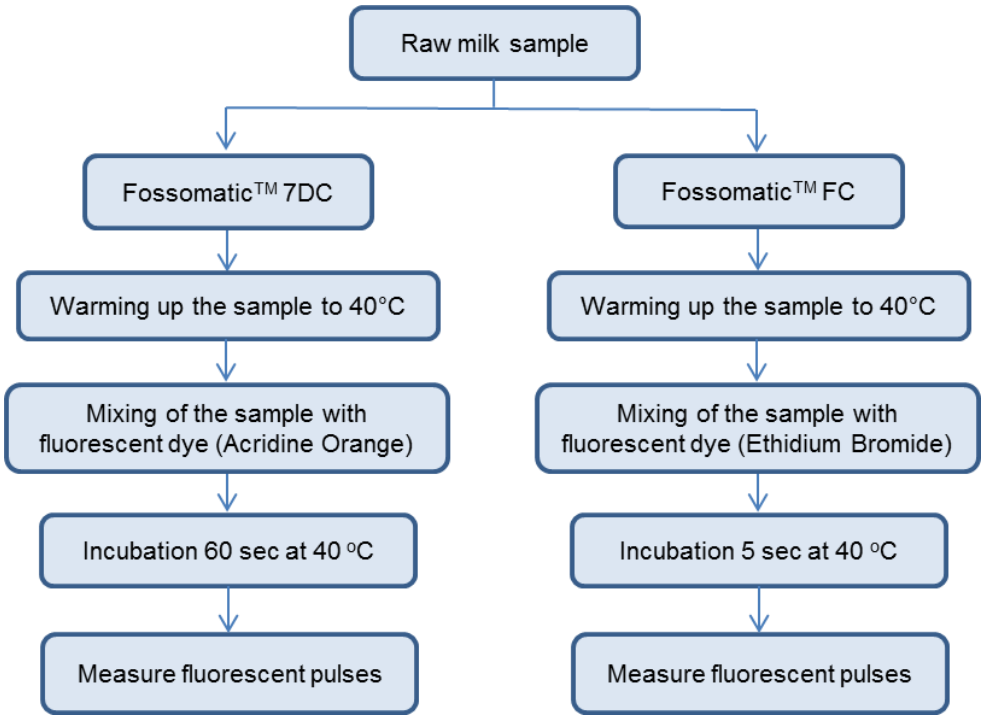
ISO 13366-1:2008 Milk - Enumeration of somatic cells - Part 1: Microscopic method (Reference method) (6).

1.5. Comparison instrument

Fossomatic™ FC with MicroVal certificate number 2015LR55.

1.6. Validation procedure

The measurement procedure with both instruments is schematically presented below, which is illustrative for the mutual resemblance:



1.7. Materials and equipment used

- Milk leucocyte suspension, prepared by creaming of raw herd bulk milk with a cell count of about 200.10^3 cells/mL and subsequent centrifugation of the cream layer. The procedure for leucocyte isolation from milk has been developed in a collaboration of Cornell University (USA) and ASIA-LSL (Italy) in 2011/2012 (7). This procedure is also advocated by the EU Joint Research Centre for the development of a certified reference material
- Preservation mixture with an end concentration in the milk of 0,02 % m/m sodium azide and 0,005% m/m bronopol
- 'Blank milk' – semi skimmed UHT milk with 1 mL/L polypropylene glycol 2000 (Baker) and 0,04 % m/m bronopol
- Stock and working solutions for Fossomatic™ 7 DC and Fossomatic™ FC, prepared according to manufacturers' instructions from supplied consumables:
    - Cleaning solution
    - Buffer solution
    - Rinse solution
    - Incubation/dye solution
    - Blank solution
- Pilot samples - preserved commingled raw milk samples with representative somatic cell count for the routine samples
- Calibration samples - a series of preserved milk samples in ascending order of adjusted somatic cell count in the range 100.10^3/mL – $2\,000.10^3$/mL, which is used in the calibration of Fossomatic™ FC. The concentrations were adjusted with the leucocyte suspension. Samples were stored at 2 - 8 ^{o}C for a maximum of 3 months
- Individual raw cow's milk samples and raw herd bulk cow's milk samples
- Flip-top disposable vials
- Pipettes
    - Adjustable pipettes with tips
    - Serological pipettes
- Standard laboratory glassware and utensils

To perform the experimental work described in this test protocol the following was needed:

- Fossomatic™ 7 DC
- Fossomatic™ FC (MicroVal certificate number 2015LR55)
- Instruction and method implementation
- Statistical expertise.

1.8. Safety precautions

Good Laboratory Practices for running food analyses were followed.

2. Methods' comparison study

2.1. Performance characteristics of the alternative method

2.1.1. Stability (according to ISO 8196-3 § 5.2.2.1.1)

The stability of the alternative method was verified by mimicking routine testing circumstances throughout a working day. To evaluate the stability of the instrument, the standard deviation of repeatability (s_r), the standard deviation of means (s_x), the standard deviation between checks (s_c) and the standard deviation of daily reproducibility ($s_{R,daily}$) were determined for different somatic cell count levels.

2.1.1.1. Measurement protocol and calculations

Preserved 'blank milk' was spiked with milk leucocyte suspension at three cell count levels: low, medium and high. The corresponding cell count ranges are given in Table 1.

Table 1. Cell count levels of samples used in the stability, repeatability and intra-laboratory reproducibility studies with the Fossomatic™ 7 DC instrument

Cell count levels	Cell counts measured with Fossomatic™ 7DC (.10^3 cells/mL)
Low	153
Medium	516
High	1 516

The spiked milk samples were stored at 2 ± 2 °C for a maximum of 1 month.

Samples from each cell count level were measured in triplicate (n=3) with the Fossomatic™ 7 DC in random order each 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 (s_r), the standard deviation of means (s_x), the standard deviation between checks (s_c) and the standard deviation of daily reproducibility ($s_{R,daily}$) were calculated according to ISO 8196-3:2009 (2). The calculations were performed without any transformation.

For every check, *j* (*j=1….q*), the mean was calculated according to:

$$\bar{x}_j = \sum x_{ij} / n$$

with n = number of measurements (n=3) and i = replicate.

and the standard deviation of replicates according to:

$$s_{rj} = \left[\sum (x_{ij} - \bar{x_j})^2/(n-1)\right]^{1/2}$$

For the whole check sequence the following parameters were calculated:
the standard deviation of repeatability (s_r)

$$s_r = \left(\sum s_{rj}^2/q\right)^{1/2}$$

with q = number of checks (q = 20)

the standard deviation of means ($s_{\bar{x}}$)

$$s_{\bar{x}} = \left[\sum (\bar{x_j} - \bar{x})^2/(q-1)\right]^{1/2} = \left\{\left[\sum \bar{x}_j^2 - \frac{\left(\sum \bar{x_j}\right)^2}{q}\right]/(q-1)\right\}^{1/2}$$

with

$$\bar{x} = \sum \bar{x_j}/q$$

the standard deviation between checks (s_c)

$$s_c = (s_{\bar{x}}^2 - s_r^2/n)^{1/2}$$

if $s_c < 0$ then $s_c = 0$

the standard deviation of daily reproducibility ($s_{R,daily}$)

$$s_{R,daily} = (s_c^2 + s_r^2)^{1/2}$$

The stability of the method response during the sequence of check tests was visualized by plotting the means of the measurement results ($\bar{x_j}$) on the y-axis, versus the check sequence numbers, on the x-axis.
The significance of a possible observed deviation or fluctuation for the standard deviation of means was tested with the *F*-test of a one-way ANOVA with α= 0,05.

2.1.1.2. Results

A summary of the stability results is given in Table 2.

Table 2. The standard deviation of repeatability (s_r), the standard deviation of means (s_x), the standard deviation between checks (s_c) and the standard deviation of daily reproducibility ($s_{R,\,daily}$) of the Fossomatic™ 7 DC per examined cell count level

Cell count levels	s_r (.10^3 cells/mL)	s_x (.10^3 cells/mL)	s_c (.10^3 cells/mL)	$s_{R,daily}$ (.10^3 cells/mL)
low (153.10^3 cells/mL)	6,6	10,8	10,1	12,1
medium (516.10^3 cells/mL)	11,6	20,9	19,8	23,0
high (1 516.10^3 cells/mL)	15,9	31,8	30,4	34,3

The standard deviation of repeatability (s_r) for each cell count level meets the requirements according to the EURL MMP document and ISO 13366-2, see Table 3.

Table 3. The standard deviation of repeatability (s_r) of the Fossomatic™ 7 DC calculated per cell count level and acceptability values according to ISO 13366-2

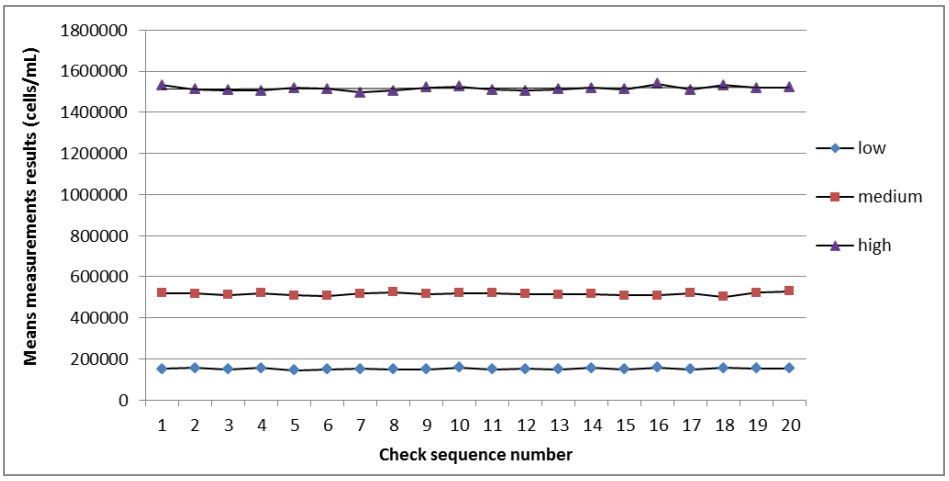
Cell count levels	s_r, calculated	s_r, acceptibility values according to ISO 13366-2*
	(.10^3 cells/mL)	
Low (153.10^3 cells/mL)	6,6	< 9,0
Medium (516.10^3 cells/mL)	11,6	< 20,3
High (1 516.10^3 cells /mL)	15,9	< 45,0
	(%)	
Low (153.10^3 cells/mL)	4	< 6
Medium (516.10^3 cells/mL)	2	< 4
High (1 516.10^3 cells /mL)	1	< 3

*the acceptability values presented in .10^3 cells/mL are calculated on the basis of the measured cell count levels and inter/extrapolation of the values in Table 2 in ISO 13366-2.

For the standard deviation of daily reproducibility ($s_{R,daily}$), standard deviation between checks (s_c) and standard deviation of means (s_x) there are no official requirements. The calculated standard deviations for each cell count level were small which demonstrated that the variation of instruments read-outs throughout the day was very small.

The plot visualizing the stability of the method response during the day is given in Figure 1.

Figure 1. Fossomatic™ 7 DC stability throughout the working day based on the means of the measurement results at three cell count levels



The significance of a possible observed deviation or fluctuation during the day was verified with the *F*-test of a one-way ANOVA. The results are given in Table 4.

Table 4. *F*- test (α=0,05) of a one-way ANOVA per cell count level

Cell count level	Source of variation	Sum of squares	Degrees of freedom	Mean of squares	*F* calculated	table values $F_{0,95}$
Low	Between groups	$7{,}0.10^2$	19	$3{,}7.10^1$		
	Within group	$1{,}7.10^3$	39	$4{,}5.10^1$		
					0,82	1,86
	Total	$2{,}4.10^3$	58			
Medium	Between groups	$2{,}6.10^3$	19	$1{,}4.10^2$		
	Within group	$5{,}4.10^3$	40	$1{,}4.10^2$		
					1,03	1,85
	Total	$8{,}0.10^3$	59			
High	Between groups	$6{,}0.10^3$	19	$3{,}2.10^2$		
	Within group	$1{,}0.10^4$	40	$2{,}5.10^2$		
					1,25	1,85
	Total	$1{,}6.10^4$	59			

The calculated F_{obs} values per cell count level were compared with the critical $F_{0,95}$ values. For all cell count levels no significant shift of instrument response was observed.

2.1.1.3. Conclusion

The Fossomatic™ 7 DC flow cytometer functions stable throughout the working day and the stability complies with the requirements of the EURL MMP document and ISO 13366-2 (3).

2.1.2. Repeatability *r* (according to ISO 8196-3 § 5.2.2.1.1)

The repeatability is the absolute difference between two independent single test results, 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.

The repeatability (*r*) is evaluated at different concentration levels.

2.1.2.1. Measurement protocol and calculations

The repeatability (*r*) of Fossomatic™ 7 DC was calculated from the stability experiment. For measurement protocol and calculations see clause 2.1.1.1. Additionally, the repeatability was calculated from testing results with 140 individual raw cow's milk samples preserved with 0,02 % m/m sodium azide and 0,005% m/m bronopol and 142 unpreserved raw herd bulk cow's milk samples representative for different somatic cell count levels as shown in Table 5. From the herd bulk cow's milk samples 17 were with elevated fat content, > 5 %. Herd bulk cow's milk samples with somatic cell count > 600.10^3 cells/mL were prepared by spiking herd bulk milk samples with milk leucocyte suspension.

Table 5. Raw cow's milk samples selected for estimation of the repeatability of the Fossomatic™ 7 DC

Cell count levels ($.10^3$ cells/mL)	Number of individual cow's milk samples	Number of herd bulk cow's milk samples
50 - 200	22	39
201 - 400	22	57
401 - 600	21	15
601 - 1 000	30	15
1 000 - 2 000	45	16
Total number of samples	140	142

All raw cow's milk samples were measured in duplicate (n= 2) with Fossomatic™ 7 DC. The standard deviation of repeatability (s_r) was calculated for the individual raw cow's milk and raw herd bulk cow's milk separately and for each cell count level as described in clause 2.1.1.1. The calculations were performed without any transformation.

The repeatability (*r*) is calculated as:

$$r = 2{,}83 s_r$$

2.1.2.2. Results

The repeatability (*r*) of Fossomatic™ 7 DC instrument was calculated from the stability experiment (clause 2.1.1.2). The results and the acceptability values are given in Table 6.

Table 6. The repeatability (*r*) of the Fossomatic™ 7 DC calculated per cell count level and acceptability values according to ISO 13366-2

Cell count levels	*r*, calculated	*r*, acceptibility values according to ISO 13366-2*
	(.10^3 cells/mL)	
Low (153.10^3 cells/mL)	18,7	< 25,5
Medium (516.10^3 cells/mL)	32,8	< 57,4
High (1 516.10^3 cells /mL)	45,0	< 127,4
	(%)	
Low (153.10^3 cells/mL)	11	< 17
Medium (516.10^3 cells/mL)	6	< 11
High (1 516.10^3 cells /mL)	3	< 9

*the acceptability values presented in .10^3 cells/mL are calculated on the basis of the measured cell count levels and inter/extrapolation of the values in Table 2 in ISO 13366-2.

The calculated repeatability (*r*) for individual raw cow's milk samples and raw herd bulk cow's milk samples measured with the Fossomatic™ 7 DC instrument and the acceptability values are presented in Table 7.

Table 7. The repeatability (*r*) of the Fossomatic™ 7 DC calculated per cell count level for individual raw cow's milk samples and bulk herd milk samples and acceptability values according to ISO 13366-2

Cell count levels (.10^3 cells/mL)	Mean level samples (.10^3 cells/mL)	*r*, individual cow's milk samples	*r*, herd bulk cow's milk samples	*r*, acceptability values ISO 13366-2
		(.10^3 cells/mL)		
50 - 200	115	13,7	14,2	< 19,6
201 - 400	257	20,2	18,9	< 37,2
401 - 600	474	33,0	20,4	< 53,4
601 - 1 000	750	37,7	31,9	< 62,6
1 000 - 2 000	1 350	58,7	36,6	< 119,6
		(%)		
50 - 200	115	12	12	< 17
201 - 400	257	8	7	< 14
401 - 600	474	7	4	< 11
601 - 1 000	750	5	4	< 8
1 000 - 2 000	1 350	4	3	< 8

*the acceptability values presented in .10^3 cells/mL are calculated on the basis of the measured cell count levels and interpolation of the values Table 2 in ISO 13366-2.

The calculated repeatability (*r*) for Fossomatic™ 7 DC is considerably lower than required by the EURL MMP document and ISO 13366-2 for all cell count levels for both individual cow's and herd bulk milk.

2.1.2.3. Conclusion

The repeatability (*r*) of the Fossomatic™ 7 DC complies with the requirements of EURL MMP document and ISO 13366-2 (3) at all cell count levels.

2.1.3. Carry-over effect (according to ISO 8196-3 § 5.2.2.1.2)

Strong differences in somatic cell count levels between two successively analysed samples may influence the result of the second one.

Differences could be caused by incomplete rinsing of the flow system and the measuring cell by liquid circulation and contamination by the stirring device. Automatic correction of results is acceptable within certain limits, provided it can be proven that there is a systematic and constant transfer of a small quantity of material from one measurement to the next. Automated analysers for liquids often allow automatic correction to compensate for the overall carry-over effect when necessary.

2.1.3.1. Measurement protocol and calculations

Preserved 'blank milk' was spiked with milk leucocyte suspension at three cell count levels, which were used as "high" samples for the evaluation of the carry-over of Fossomatic™ 7 DC. The carry-over was evaluated per cell count level separately. The cell count levels of the "high" samples are given in Table 8. The "low" samples were unspiked 'blank milk'.

Table 8. Cell count levels of the "high" samples used in the carry-over assessment of Fossomatic™ 7 DC

Cell count levels of the "high" samples	Theoretical ($.10^3$ cells/mL)	Measured ($.10^3$ cells/mL)
High 1	500	527
High 2	1 000	1 002
High 3	3 000	2 942

The spiked milk samples were stored at 2 ± 2 °C for a maximum of 1 month.

Fossomatic™ 7 DC measurements were performed without carry-over correction factor on 20 sets of samples per cell count level with the following sequence:

$$(L_{L_1}, L_{L_2}, L_{H_1}, L_{H_2})_1, (L_{L_1}, L_{L_2}, L_{H_1}, L_{H_2})_2 \ldots (L_{L_1}, L_{L_2}, L_{H_1}, L_{H_2})_{20}.$$

thus,

(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 calculations were performed on raw data without any transformation. The carry over (CO) was obtained by applying the following equations:

$$C_{H/L} = \frac{(\sum L_{L_1} - \sum L_{L_2}) \times 100}{(\sum L_{H_2} - \sum L_{L_2})} = (\overline{L_{L_1}} - \overline{L_{L_2}}) \times 100 / (\overline{L_{H_2}} - \overline{L_{L_2}})$$

$$C_{L/H} = \frac{(\sum L_{H_2} - \sum L_{H_1}) \times 100}{(\sum L_{H_2} - \sum L_{L_2})} = (\overline{L_{H_2}} - \overline{L_{H_1}}) \times 100 / (\overline{L_{H_2}} - \overline{L_{L_2}})$$

The carry over effect should not exceed the limit of 2 % as required in the EURL MMP document.

2.1.3.2. Results

For each cell count level the ratios $C_{H/L}$ and $C_{L/H}$ were calculated. The results are given in Table 9.

Table 9. Calculated ratios $C_{H/L}$ and $C_{L/H}$ per cell count level

Cell count levels of the "high" samples	Calculated $C_{H/L}$ (%)	Calculated $C_{L/H}$ (%)
High 1 (ca. 500.10^3 cells/mL)	1,49	0,00
High 2 (ca. 1 000.10^3 cells/mL)	0,05	0,12
High 3 (ca. 3 000.10^3 cells/mL)	1,44	0,64

The calculated relative carry-over effect for each cell count level was smaller than the limit CO < 2 %.

2.1.3.3. Conclusion

The carry-over effect with measurements on the Fossomatic™ 7 DC complies with the requirements in EURL MMP document (3), CO < 2 %, for each cell count level.

2.1.4. Linearity (according to ISO 8196-3 §5.2.2.1.3 and ISO 13366-2 §6.2.2)

According to the classical definition of an indirect method, the instrument signal should result from a characteristic of the component measured and thereby allow the definition of a simple relationship to the component concentration. Linearity expresses the constancy of the ratio between the increase in the concentration of a component and the corresponding increase of the alternative method result. Therefore, linearity of the measurement signal is in most cases essential to maintain a constant sensitivity over the measuring range and to allow easy handling of calibration and fittings. Moreover, it allows in routine (to some extent) measurements beyond the calibration range through linear extrapolation.

2.1.4.1. Measurement protocol and calculations

To evaluate linearity, two sets of samples with cell count levels distributed over the range of 0 – 10 000.10^3 cells/mL were prepared. Preserved 'blank milk' was spiked with milk leucocyte suspension in steps of 150.10^3 cells/mL in the range 0 – 2 000.10^3 cells/mL, covering the working range in routine testing, and in steps of 1 000.10^3 cells/mL in the range 2 000.10^3 - 10 000.10^3 cells/mL. The samples in the first set were measured 4 times in the order of increasing cell count and in the second set 4 times in the order of decreasing cell count. Per sample in total 8 results were collected.

The ratio r_C was calculated as the ratio of the residual range to the signal value range[1]. The calculated cell count levels of the spiked samples were used as the reference values for the calculations.
The means of the replicates per sample (n = 8) were calculated. The mean results were processed by linear regression:

$$y = bx + a$$

y = instrument value (measured value),
x = calculated reference value of the spiked samples.

The residuals, e_i, were calculated from the means of replicates and the theoretical reference:

$$e_i = y_i - (bx_i + a)$$

The linearity was visually inspected by plotting the residuals, e_i, on the y-axis and the theoretical concentrations on the x-axis.

The relative linearity bias was expressed with the ratio r_C:

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

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

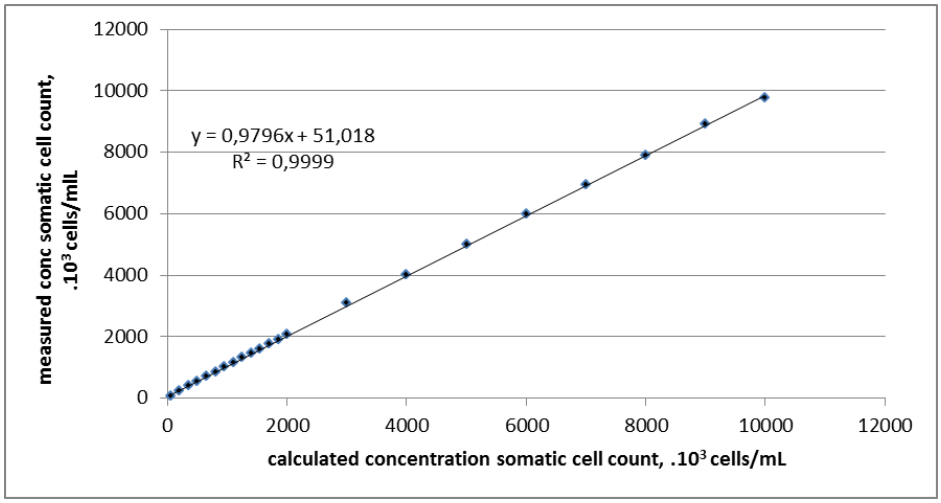
The ratio r_C should be below 2% in order to comply with the EURL MMP document and ISO 13366-2.

2.1.4.2. Results

The results appeared to be linear in the whole testing range up to 10 000.10[3] cells/mL with r_C= 1,70 %.
The results are pictured in Figure 2.

[1] The ratio r_C is calculated by using the formula described in ISO 13366-2. The symbols are as in the original formula and deviate from these used in ISO 8196-3.

Figure 2. Linearity of FossomaticTM 7 DC in the testing range up to 10 000.10^3 cells/mL



The Fossomatic^{TM} 7 DC also appeared to be linear (r_C= 1,08 %) when more specifically examined in the performance range 100 – 1 500.10^3 cells/mL.

2.1.4.3. Conclusions

The instrument is linear in the normal working range and in the wider measurement range up to 10 000.10^3 cells/mL. In both ranges, the linearity of the Fossomatic^{TM} 7 DC complies with the stated maximum limit value of r_C ≤ 2 % in the EURL document and ISO 13366-2 (3).

2.1.5. Limits of quantification (according to ISO 8196-3 §5.2.2.1.5 and §5.2.2.1.6)

Limits of a measurement with an instrumental method exist at both extremities of the analytical range, e.g. a lower limit and an upper limit. The assessment of the measurement limits can be carried out in combination with the evaluation of the linearity. If linearity is not achieved throughout the whole concentration range, then the actual range of application for the method should be evaluated.

The lower limit of quantification is the smallest amount of measurand that can be measured and quantified with a defined coefficient of variation, CV. The lower limit of quantification is defined as multiples of the standard deviation, σ, of random error observed near zero (blank).

The upper limit of quantification corresponds to the threshold where the signal deviates significantly from linearity.

2.1.5.1. Measurement protocol and calculations

2.1.5.1.1. Lower limit of quantification, L_Q

Semi skimmed UHT milk was measured 20 times with Fossomatic^{TM} 7 DC. The mean and standard deviation, σ, of the measurements were calculated and the lower limit of quantification, $L_{Q,}$ was calculated as:

$$L_Q = mean + 10\sigma$$

2.1.5.1.2. Upper limit of quantification

The upper limit of quantification is the highest possible reading of the method without interference of methodological limitations. The upper limit of quantification of the alternative method is the ratio, r_C, exceeding the 2 % limit value according to EURL MMP document and ISO 13366-2.

The upper limit of quantification of Fossomatic™ 7 DC was determined as linearity of the instrument in the range above the working range. For measurement protocol and calculations see clause 2.1.4.

2.1.5.2. Results

2.1.5.2.1. Lower limit of quantification, L_Q

The obtained results for determining the lower limit of quantification are shown in Table 10.

Table 10. Results lower limit of quantification of the Fossomatic™ 7 DC

Measurement	Result (.10^3 cells/mL)
1	0
2	7
3	1
4	0
5	0
6	1
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
Mean	0,5
σ	1,6
L_Q	16,2

The resulting lower limit of quantification is 16,2.10^3 cells/mL.

2.1.5.2.2. Upper limit of quantification

The results appeared to be linear in the range up to 10 000.10^3 cells/mL with r_C= 1,70 %. The results are pictured in Figure 2.

The upper limit of quantification of Fossomatic™ 7 DC complies with the EURL MMP requirement of >1 400.10^3 cells/mL.

2.1.5.3. Conclusion

The lower limit of quantification of Fossomatic™ 7 DC is 16.10^3 cells/mL according to ISO 8196-3.
The upper limit of quantification of Fossomatic™ 7 DC is at least 10 000.10^3 cells/mL and complies with EURL requirements.

2.1.6. Evaluation of factors affecting the results (according to ISO 13366-2 §10.2 and EURL MMP document)

High fat and protein content in the milk could potentially interfere with somatic cell count measurements on the Fossomatic™ 7 DC. The influence of fat and protein content was examined at three relevant levels within the range of the measurand by applying linear regression analysis.

2.1.6.1. Measurement protocol and calculations

The somatic cell count in preserved raw cow's milk with 3, 6 and 8 % fat and preserved raw cow's milk with 3,5, 4,5 and 5,5 % protein content was adjusted at five cell count levels.
The spiked milk samples were stored at 2 ± 2 °C for a maximum of 1 month.
Each sample was analysed four times with Fossomatic™ 7 DC.
The means of the replicate measurements per sample (n=4) were calculated. The possible interference of high fat and protein content on somatic cell counting was assessed by linear regression of the mean instrument values at each component concentration level against the calculated values:

$$y = bx + a$$

y = instrument value,
x = calculated value of the spiked samples.

Differences in obtained slopes and intercepts are indicative for interference of high fat and protein content with the somatic cell count. It was required that slopes are within the 95 % confidence limit interval of the calculated slope for samples with 3 % fat and 3,5 % protein or that there is an overlap between the 95% confidence limit intervals.
The relative linearity bias per fat and protein concentration count was expressed with the ratio r_C and was calculated as described in clause 2.1.4.1.
Additionally 17 raw bulk cow's milk samples with fat content > 5 % were included in the analysis as described in clause 2.2.

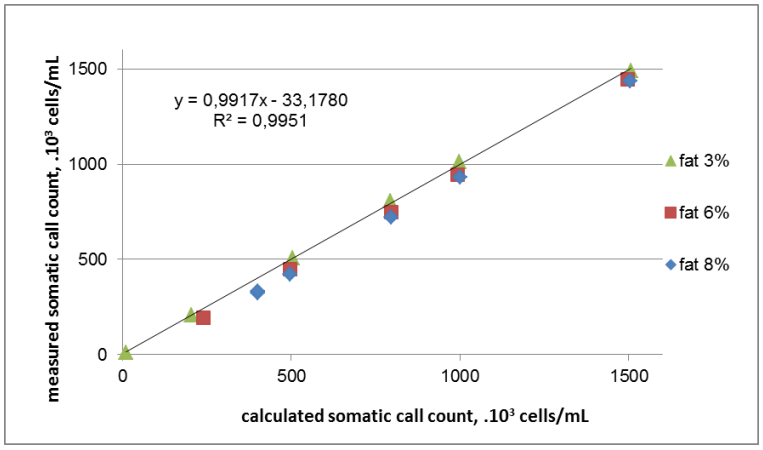
2.1.6.2. Results

The calculated slope (*b*), intercept (*a*), 95% confidence limit interval from linear regression analysis, linearity ratio (r_C) and standard error of accuracy (s_{yx}) on results obtained with the Fossomatic™ 7 DC on milk samples with different fat content and different somatic cell count levels are given in Table 11 and visualisation of the results is shown in Figure 3.

Table 11. Calculated slope (*b*), intercept (*a*), 95% confidence limit interval from linear regression analysis, linearity ratio (r_C) and standard error of accuracy (s_{yx}) on results obtained with the Fossomatic™ 7 DC on milk samples with different fat content and different somatic cell count levels

Fat concentration	slope (*b*)			intercept (*a*) (.10^3 cells/mL)			r_C	s_{yx}
	calculated	lowest 95%	highest 95%	calculated	lowest 95%	highest 95%	(%)	(%)
3%	0,9935	0,9704	1,0166	7,1754	-12,1478	26,4986	1,5	1,5
6%	0,9960	0,9904	1,0017	-45,8040	-50,5418	-41,0662	0,4	0,3
8%	1,0056	0,9915	1,0197	-75,7240	-87,8844	-63,5637	1,2	0,7

Figure 3. Linearity of the results obtained with Fossomatic™ 7 DC on milk samples with increasing fat content and different somatic cell count levels



The slope and intercept for each fat level was calculated using linear regression. The slopes, intercepts and the 95 % confidence intervals obtained with milk samples containing 6 % and 8 % fat were compared with the 95 % confidence limit interval of the slope and intercept obtained with milk samples containing 3 % fat (Table 11). The slopes obtained with 6 % and 8 % fat content were within the 95 % confidence interval for the slope of milk with 3 % fat. The calculated intercepts however were outside the 95 % confidence interval of milk with 3 % fat. To evaluate the impact of the deviating intercept, additional statistical analyses were performed. The normal distribution of the results was evaluated with the Shapiro test (8) and the standard deviations were compared applying Bartlett's test (9). It was concluded that the deviation observed in the intercept indicates that fat could cause some noise in the results but does not influence the somatic cell count results. The respective report is separately provided for MicroVal's evaluation (10).

The calculated linearity ratio r_C for each fat concentration was lower than 2 %. The results obtained with Fossomatic™ 7 DC on milk samples with increasing fat content and different somatic cell count levels appear to be linear up to 1 500.10^3 cells/mL.

Accuracy was calculated as standard error (s_{yx}) and compared to the requirement of ≤ 10 % in ISO 8196-3. For all levels s_{yx} was below 10 % (Table 11).

Additionally, the results obtained on 17 raw bulk milk samples with elevated fat content > 5 % were analysed with linear regression as described in clause 2.2. The variation of these results was within the variation of the results obtained on milk with lower fat content (Figure 5), indicating no interference of the higher fat content on the somatic cell count.

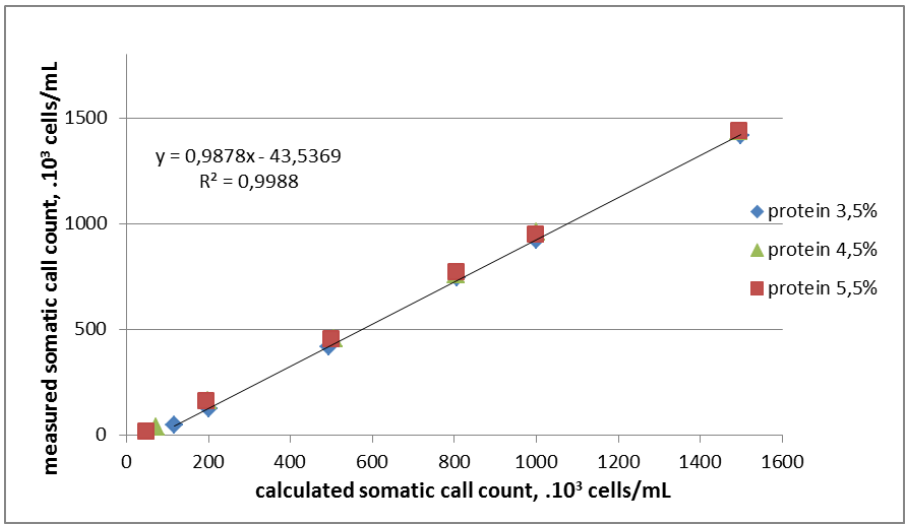
It was therefore concluded that milk fat content up to 8 % does not have a relevant influence on somatic cell count results obtained with Fossomatic™ 7 DC.

The calculated slope (*b*), intercept (*a*), 95 % confidence limit interval from linear regression analysis, linearity ratio (r_C) and standard error of accuracy (s_{yx}) on results obtained with Fossomatic™ 7 DC on milk samples with different protein content and different somatic cell count levels are given in Table 12 and visualisation of the results is shown in Figure 4.

Table 12. Calculated slope (*b*), intercept (*a*), 95% confidence limit interval from linear regression analysis, linearity ratio (r_C) and standard error of accuracy (s_{yx}) on results obtained with the Fossomatic™ 7 DC on milk samples with different protein content and different somatic cell count levels

Protein concentration	slope (*b*)			intercept (*a*) (.10³ cells/mL)			r_C (%)	s_{yx} (%)
	calculated	lowest 95%	highest 95%	calculated	lowest 95%	highest 95%		
3,5%	0,9946	0,9769	1,0124	-70,1604	-84,9854	-55,3354	1,3	1,1
4,5%	0,9868	0,9747	0,9989	-34,7114	-44,8298	-24,5929	0,9	0,8
5,5%	0,9834	0,9683	0,9985	194,0000	-39,2834	-14,1172	1,3	1,1

Figure 4. Linearity of the results obtained with the Fossomatic™ 7 DC on milk samples with different protein content and different somatic cell count levels



The slope and intercept for each protein level was calculated using linear regression. The slopes, intercepts and the 95 % confidence limit intervals obtained with milk samples containing 4,5 % and 5,5 % protein were compared with the 95 % confidence limit interval of the slope and intercept obtained with milk samples containing 3,5 % protein (Table 12). The slopes obtained with milk samples containing 4,5 % and 5,5 % protein were within the 95% confidence interval for the slope obtained with

3,5 % protein. The calculated intercepts however were outside the 95 % confidence interval of milk with 3,5 % protein. To evaluate the impact of the intercept additional statistical analysis was performed. As with the possible influence of the fat content, the normal distribution of the results was evaluated with the Shapiro test (8) and the standard deviations were compared applying Bartlett's test (9). It was concluded that the deviation observed in the intercept indicates that protein could cause some noise in the results but does not influence the somatic cell count results. The respective report is separately provided for MicroVal's evaluation (10).

The calculated linearity ratio r_C for all tested protein concentrations was lower than 2 %. The results obtained with Fossomatic™ 7 DC on milk samples with increasing protein content and different somatic cell count levels appear to be linear up to 1 500.10^3 cells/mL.

Furthermore, the accuracy was calculated as standard error (s_{yx}) and compared to the requirement of ≤ 10 % in ISO 8196-3. For all levels was s_{yx} was below 10 % (Table 12).

It was therefore concluded that milk protein content up to 5,5 % does not have a relevant influence on somatic cell count results obtained with Fossomatic™ 7 DC.

2.1.6.3. Conclusions

No relevant influence of elevated fat and protein content of the milk was observed on the somatic cell count results obtained with the Fossomatic™ 7 DC.

2.2. Comparison of Fossomatic™ 7 DC and Fossomatic™ FC

2.2.1. Measurement protocol and calculations

The intra-laboratory reproducibility is the absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by possibly different operators using different instruments at different times (within at most a few hours). The intra-laboratory reproducibility ($R_{intra\text{-}lab}$) of the Fossomatic™ 7 DC was evaluated at different somatic cell count levels through comparison with the Fossomatic™ FC. $R_{intra\text{-}lab}$ was calculated with 144 individual raw cow's milk samples preserved with 0,05 % m/m bronopol and 0,005 % m/m kathon and 142 unpreserved raw herd bulk cow's milk samples as shown in Table 13. The herd bulk cow's milk samples with somatic cell count > 600.10^3 cell/mL were artificially prepared by spiking with milk leucocyte suspension.

Table 13. Raw cow's milk samples selected for estimation of the intra-laboratory reproducibility of the Fossomatic™ 7 DC and Fossomatic™ FC

Cell count levels (.10^3 cells/mL)	Number of individual cow's milk samples	Number of herd bulk cow's milk samples
50 - 200	36	39
201 - 400	34	57
401 - 600	28	15
601 - 1 000	25	15*
1 000 - 2 000	21	16*
Total number of samples	144	142

*bulk milk spiked with milk leucocyte suspension

The samples were measured in random order with Fossomatic™ 7 DC and Fossomatic™ FC. Both instruments were operating in the routine laboratory of Qlip. The time between the measurements on both instruments did not exceed 2 hours. Different laboratory technicians have operated the instruments.

The standard deviation of reproducibility ($s_{R\ intra\text{-}lab}$) was calculated for the individual raw cow's milk and raw herd bulk cow's milk separately and for each cell count level. The calculations were performed without any transformation.

The standard deviation of intra-laboratory reproducibility, $s_{R\ intra\text{-}lab}$, was calculated as:

$$s_{R\ intra-lab} = \sqrt{\frac{\sum(x_1 - x_2)^2}{2n}}$$

where

x_1 - single result obtained with the Fossomatic™ 7 DC

x_2 - single result obtained with the Fossomatic™ FC

n - number of samples.

The intra-laboratory reproducibility, $R_{intra\text{-}lab}$, was calculated as:

$$R_{intra-lab} = 2{,}83.s_{R,intra-lab}$$

The relationship between results with the evaluated instrument models was visually inspected by plotting the results obtained with the Fossomatic™ 7 DC on the y-axis and the results obtained with the Fossomatic™ FC on the x-axis. The standard error (s_{yx}) was calculated.

The accuracy of Fossomatic™ 7 DC against Fossomatic™ FC was evaluated by linear regression analysis after natural logarithmic transformation of the results. The results were considered equivalent when the calculated slope and intercept did not differ significantly from these of the identity function

(f(x) = x), which means slope = 1 and intercept = 0 are within the 95 % confidence limit interval of the calculated slope and intercept.

2.2.2.Results

The intra-laboratory reproducibility results and the acceptability values are given in Table 14.

Table 14. Intra-laboratory reproducibility R_{intra} and the acceptability values according to ISO 13366-2

Cell count levels ($.10^3$ cells/mL)	Mean level samples ($.10^3$ cells/mL)	$R_{intra-lab}$, individual cow's milk samples	$R_{intra-lab}$, herd bulk cow's milk samples	$R_{intra-lab}$, acceptability values ISO 13366-2
		($.10^3$ cells/mL)		
50 - 200	130	19,3	25,1	< 26,0
201 - 400	265	28,5	42,5	< 45,0
401 - 600	485	44,6	60,9	< 67,9
601 - 1 000	750	70,7	152,9*	< 82,5
1 000 - 2 000	1 350	128,3	284,9*	< 148,5
		(%)		
50 - 200	130	15	19	< 20
201 - 400	265	11	16	< 17
401 - 600	485	9	13	< 14
601 - 1 000	750	9	20*	< 11
1 000 - 2 000	1 350	10	21*	< 11

*bulk milk spiked with milk leucocyte suspension

The calculated intra-laboratory reproducibility of Fossomatic™ 7 DC complies with the ISO 13366-2 acceptability values for each cell count level for individual cow´s milk and in the range up to 600.10^3 cell/mL for the herd bulk cow´s milk samples. The deviations observed in the reproducibility obtained with herd bulk milk with somatic cell count > 600.10^3 cell/mL were explained with the type of the samples. Herd bulk cow's milk with such high somatic cell count were artificially prepared by spiking with milk leucocyte suspension. The intra-laboratory reproducibility values for herd bulk milk were higher than these obtained for individual cow's milk samples. This observation could possibly be explained by the age of the samples: ca. 72 hours old herd bulk milk samples, and ca. 48 hours old individual cow's milk samples. The milk samples should be analysed within 72 hours maximum in order to get reliable results according to the manufacturer's specifications.

The accuracy of Fossomatic™ 7 DC was evaluated against Fossomatic™ FC with a linear regression. The correlation between the evaluated models is visualised in Figure 5 and Figure 6 (a and b).

Figure 5. Relationship between Fossomatic™ 7 DC and Fossomatic™ FC for individual raw cow's milk samples, preserved with 0,05 % m/m bronopol and 0,005 % m/m kathon

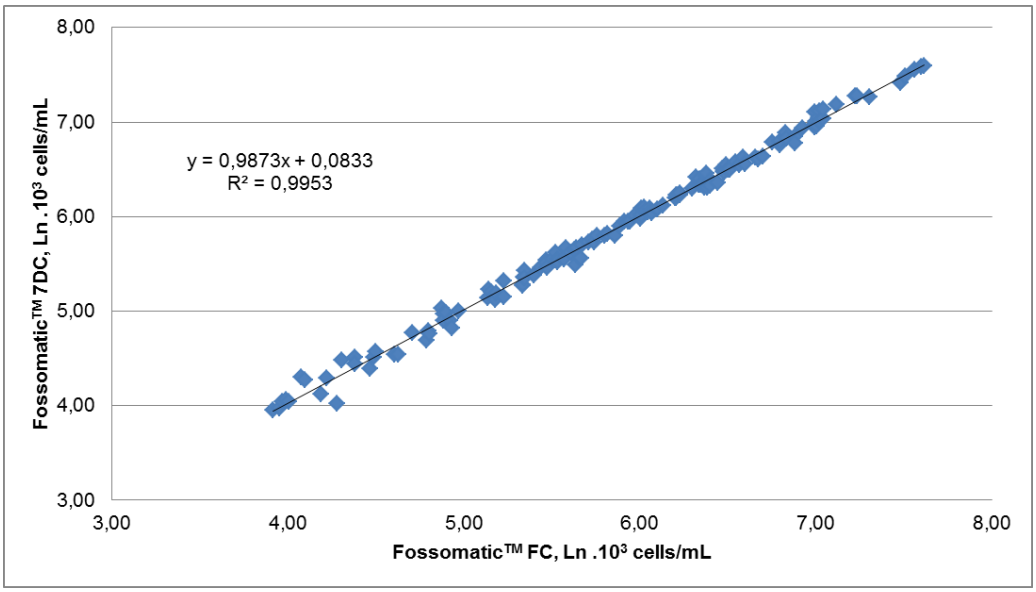


Figure 6a. Relationship Fossomatic™ 7 DC and Fossomatic™ for unpreserved raw herd bulk cow's milk samples in the range up to 2 000.10³ cell/mL

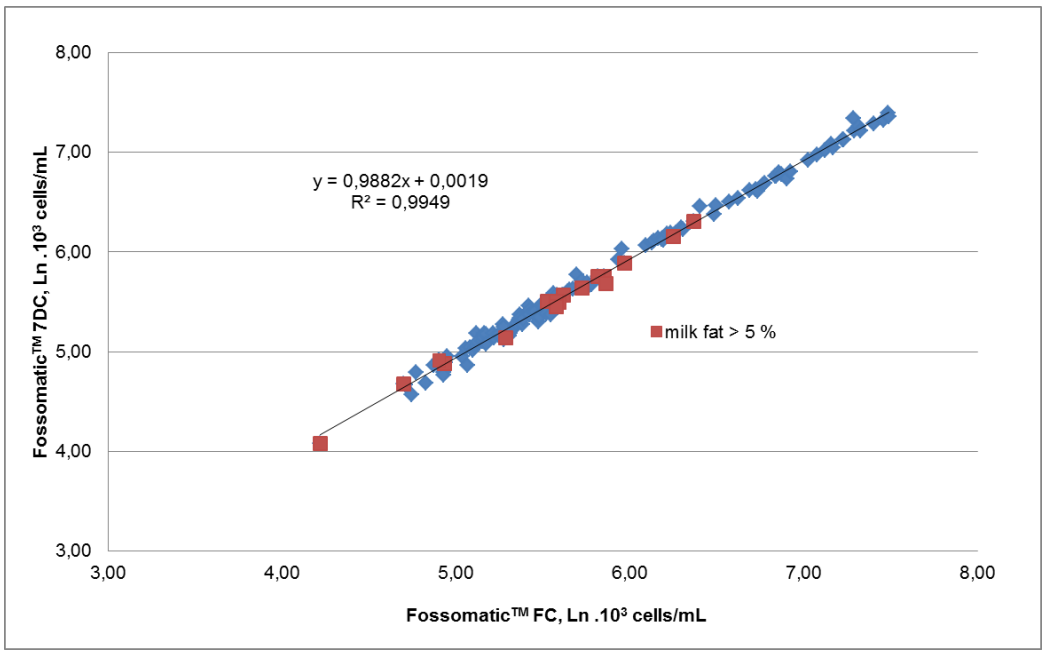
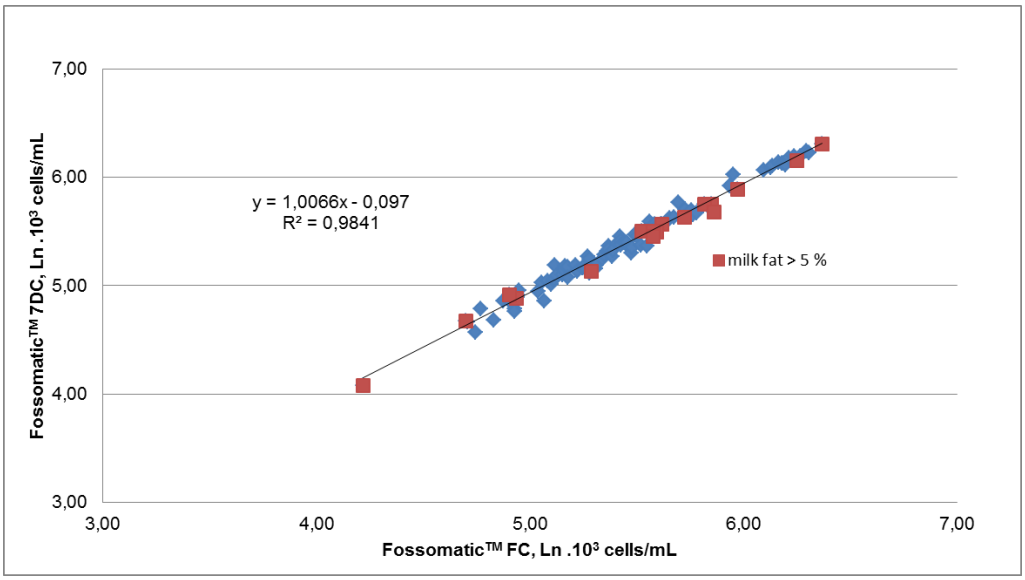


Figure 6b. Relationship Fossomatic™ 7 DC and Fossomatic™ for unpreserved raw herd bulk cow's milk samples in the range up to 600.10³ cell/mL



The slope, intercept and calculated 95 % confidence interval of the regression analysis are shown in Table 15.

Table 15. Slope, intercept and 95 % confidence interval limits from the linear regression analysis between results obtained with Fossomatic™ 7 DC and Fossomatic™ FC on raw individual cow's milk preserved with 0,05 % m/m bronopol and 0,005 % m/m kathon and unpreserved raw herd bulk cow's milk samples

	Coefficient	Lowest 95 %	Highest 95 %
Individual cow's milk samples, range up to 2 000.10³ cells/mL			
slope	0,9873	0,9760	0,9985
intercept	0,0833	0,0165	0,1501
Herd bulk cow's milk samples, range up to 2 000.10³ cells/mL			
slope	0,9882	0,9763	1,0001
intercept	0,0019	-0,0675	0,0713
Herd bulk cow's milk samples, range up to 600.10³ cells/mL			
slope	1,0066	0,9823	1,0309
intercept	-0,0970	-0,2302	0,0361

For individual cow's milk samples preserved with 0,05 % m/m bronopol and 0,005 % m/m kathon the theoretical slope = 1 and intercept = 0 were just outside the 95 % confidence limit intervals of the calculated slope and intercept. However, the dispersion between the results obtained with Fossomatic™ 7 DC and Fossomatic™ FC was small which resulted in narrow limits of the calculated 95 % confidence intervals.

The calculated standard error of the results was small, s_{yx} = 0,06 Ln.10³ cells/mL.

For unpreserved herd bulk cow's milk samples the 95 % confidence limit intervals of the calculated slope and intercept included respectively 1 and 0 for the samples up to 600.10^3 cell/mL as well as when including the samples in the higher range, meaning that the results obtained with Fossomatic™ 7 DC and Fossomatic™ FC were statistically equivalent at threshold $p < 0,05$. The calculated standard error of the results for both ranges was small, s_{yx} = 0,05 Ln.10^3 cells/mL. The samples spiked with milk leucocyte suspension did not effect the linear relationship and the accuracy of the instruments.

The small standard error (s_{yx}) and the non-significant deviation of the regression line from the identity function demonstrated a close correlation between the results obtained with both instruments on unpreserved and bronopol-based preserved raw milk samples. For these samples Fossomatic™ 7 DC and Fossomatic™ FC can be considered equivalent.

During the comparison procedure it was noted that the condition of the cells and the preservation of the samples could effect the equivalence of the results obtained with Fossomatic™ 7 DC and Fossomatic™ FC. Evaluation of individual cow's milk samples preserved with 0,02 % m/m sodium azide and 0,005% m/m bronopol showed intra-laboratory reproducibility results which did not comply with the requirement of ISO 13366-2.

2.2.3. Conclusion

The results obtained with Fossomatic™ 7 DC are equivalent to the results obtained with Fossomatic™ FC at all cell count levels when applied on unpreserved milk samples and bronopol preserved samples. The use of sodium azide as a preservative can affect the equivalence of the results obtained with both models.

3. Conclusions of the comparison study

Fossomatic™ 7 DC performance characteristics determined according to ISO 8196-3 and ISO 13366-2 are:

- Fossomatic™ 7 DC functions stable through the working day
- Repeatability (r) per cell count level:
  - Low (ca. 181.10^3 cells/mL) 11 % *(ISO 13366-2: ≤ 17 %)*
  - Medium (ca. 563.10^3 cells/mL) 6 % *(ISO 13366-2: ≤ 11 %)*
  - High (ca. 1 583.10^3 cells/mL) 3 % *(ISO 13366-2: ≤ 9 %)*
- Carry-over per cell count level (*ISO 13366-2: for each cell count level CO < 2 %)*
  - Low (ca. 500.10^3 cells/mL) $C_{H/L}$ = 1,49 %
    $C_{L/H}$ = 0,00 %
  - Medium (ca. 1 000.10^3 cells/mL) $C_{H/L}$ = 0,05 %
    $C_{L/H}$ = 0,12 %

- High (ca. 3 000.10^3 cells/mL) $C_{H/L}$ = 1,44 %
  $C_{L/H}$ = 0,64 %
- Linearity (r_C): 1,7 % *(ISO 13366-2: $r_C \leq 2$ %)*
- Lower limit of quantification (L_Q): 16.10^3 cells/mL
- Upper limit of quantification: 10 000.10^3 cells/mL
- High fat (up to 8 %) and protein (up to 5,5 %) content of the milk do not relevantly influence the somatic cell count results with the Fossomatic™ 7 DC.

Conclusions of the comparison of Fossomatic™ 7 DC and Fossomatic™ FC

The results obtained from the comparison of Fossomatic™ 7 DC and Fossomatic™ FC are:

- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in bronopol preserved individual raw cow's milk:
  - Cell level 50-200.10^3 cells/mL 15 % *(ISO 13366-2: ≤ 20 %)*
  - Cell level 201-400.10^3 cells/mL 11 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401-600.10^3 cells/mL 9 % *(ISO 13366-2: ≤ 14 %)*
  - Cell level 601-1 000.10^3 cells/mL 9 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 1 000-1 500.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 11 %)*
- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in:

  Unpreserved raw herd bulk cow's milk
  - Cell level 50-200.10^3 cells/mL 19 % *(ISO 13366-2: ≤ 20 %)*
  - Cell level 201-400.10^3 cells/mL 16 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401-600.10^3 cells/mL 13 % *(ISO 13366-2: ≤ 14 %)*

  Unpreserved raw herd bulk cow's milk spiked with milk leucocyte suspension
  - Cell level 601-1 000.10^3 cells/mL 20 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 1 000-1 500.10^3 cells/mL 21 % *(ISO 13366-2: ≤ 11 %)*
- Standard error (s_{yx}) of the results was:
  - for bronopol preserved individual raw cow's milk, s_{yx} = 0,06 Ln.10^3 cells/mL
  - for unpreserved raw herd bulk cow's milk, s_{yx} = 0,05 Ln.10^3 cells/mL
- A small significant, but irrelevant, deviation of the regression line from the identity function was observed. Close correlation was demonstrated between the results obtained with both instruments on unpreserved and bronopol preserved raw milk samples
- Results obtained with Fossomatic™ 7 DC and Fossomatic™ FC are equivalent for all cell count levels when applied on unpreserved and bronopol-preserved cow's milk samples. The use of sodium azide as a preservative can effect the equivalence of the results obtained with both models (e.g., high correlation but inter-laboratory reproducibility slightly beyond ISO requirements).

4. Final conclusion methods' comparison study

The final conclusion of the validation study is:

The Method Comparison Study of Fossomatic™ 7 DC (FOSS Analytical A/S) and the direct comparison with Fossomatic™ FC (MicroVal certificate 2015LR55) show that the results obtained with both instruments are equivalent with unpreserved and bronopol-preserved cow's milk samples. All results of the tests performed in this study confirm that the new method complies with the criteria of the EURL MMP document.

5. References

1. EURL MMP document - Criteria for the validation of instrumental (epifluorescent) methods for the enumeration of somatic cells in raw cow's milk, version 2, 21/01/2013.

2. 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.

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