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MICROVAL VALIDATION REPORT

Comparative Study

MicroVal Project 2016LR64

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

Confidential

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Summary

BacSomatic™ is a new instrument allowing the simultaneous determination of somatic cell count and total bacterial count in raw milk. However, the instrument can be used as a stand-alone instrument for either somatic cell count or total bacteria count determination as well. This MicroVal validation report presents the results obtained with the newly developed BacSomatic™ (FOSS Analytical A/S) for enumeration of somatic cells. The method comparison study for the validation of BacSomatic™ was performed against the criteria in the EURL MMP document "Criteria for the validation of instrumental (epifluorescent) methods for the enumeration of somatic cells in raw cow's milk" from January 2013 (1) and the accuracy of the instrument was evaluated as comparison with the already approved Fossomatic™ FC (MicroVal certificate 2015LR55). The results of the validation of BacSomatic™ for determination of total bacterial count are presented in a separate report (MicroVal Project 2016LR65).

Conclusions from the method comparison study

BacSomatic™ performance characteristics determined according to ISO 8196-3 and ISO 13366-2 are:

- BacSomatic™ functioned 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) 6 % *(ISO 13366-2: ≤ 8 %)*
- Carry-over (C) per cell count level (*ISO 13366-2: for each cell count level C < 2 %)*
  - Low (ca. 500.10^3 cells/mL) $C_{H/L}$ = 0,10 %; $C_{L/H}$ = 1,24 %
  - Medium (ca. 1 000.10^3 cells/mL) $C_{H/L}$ = 0,15 %; $C_{L/H}$ = 0,91 %
  - High (ca. 3 000.10^3 cells/mL) $C_{H/L}$ = 0,09 %; $C_{L/H}$ = 0,18 %
- Linearity (r_C): 1,4 % *(ISO 13366-2: r_C ≤ 2 %)*
- Lower limit of quantification (L_Q): 7.10^3 cells/mL
- Upper limit of quantification: 10 000.10^3 cells/mL
- Evaluation of factors that possibly interfere with somatic cell count results:
  High fat (up to 8 %), protein (up to 5,5 %) content and total bacterial count (up to 8.10^5 cfu/mL) of the milk did not relevantly influence the somatic cell count results determined with the BacSomatic™.

Conclusions from the comparison of BacSomatic™ and Fossomatic™ FC

The results obtained from the comparison of BacSomatic™ and Fossomatic™ FC were:

- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in individual raw cow's milk:
  - Cell level 50 - 200.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 20 %)*
  - Cell level 201 - 400.10^3 cells/mL 13 % *(ISO 13366-2: ≤ 17 %)*

- Cell level 401 – 1 000.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 11 %)*
- Cell level 1 001 - 2 000.10^3 cells/mL 6 % *(ISO 13366-2: ≤ 11 %)*

- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in 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 11 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401 – 600.10^3 cells/mL 6 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 601 - 2 000.10^3 cells/mL 11 % *(ISO 13366-2: ≤ 11 %)*
- The 95 % confidence limit interval of the slope and the intercept of the results obtained from individual raw cow's milk and the natural raw herd bulk cow's milk samples included respectively 1 and 0
- Standard error (s_{yx}) of the results was small:
  - for individual raw cow's milk s_{yx}= 0,05 Ln.10^3 cells/mL
  - for raw herd bulk cow's milk s_{yx}= 0,06 Ln.10^3 cells/mL

It is concluded that the results obtained with BacSomatic™ and Fossomatic™ FC are equivalent for all cell count levels.

Final conclusion of the validation study

The final conclusion of the validation study is:

All results obtained during the Method Comparison Study of BacSomatic™ comply with the criteria of the EURL MMP document. The Direct Comparison of results from BacSomatic™ and Fossomatic™ FC (MicroVal certificate 2015LR55) revealed equivalence in terms of enumeration of somatic cells and do comply with the criteria of the EURL MMP document.

CONTENTS

1. Introduction

The BacSomatic™ from FOSS Analytical A/S is a newly developed low-throughput instrument based on Fossomatic™ FC and BactoScan™ FC/FC+ technology, which were both recently granted with MicroVal certificates. BacSomatic™ flow cytometer is a dedicated instrument for simultaneous determination of somatic cell count and total bacterial count in raw milk. However, the instrument can also be used as a stand-alone for determination of either somatic cell or total bacteria count. This report concerns the validation of BacSomatic™ for somatic cell count in raw cow's milk.

Since independent validation is a critical success factor for the acceptance of the BacSomatic™ 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 BacSomatic™ 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 internationally-accepted performance criteria in ISO 8196-3 (2) and ISO 13366-2 (3).

BacSomatic™ is a downscaled version of Fossomatic™ FC instruments for somatic cell counting in raw milk. The Fossomatic™ FC was granted with a MicroVal certificate (2015LR55). The hardware and calculation algorithms of both models are highly similar, however the new analyser has some minor differences (using a laser as a light source instead of a halogen lamp) when compared with the Fossomatic™ FC.

The performance characteristics of BacSomatic™ for somatic cell counting are demonstrated during the method 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 method comparison study as prescribed in the EURL MMP document from January 2013 and results of comparison of BacSomatic™ and Fossomatic™ FC somatic cell count measurement using routine samples.

1.1. Principle of the alternative method

The BacSomatic™ is a low-throughput 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, 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 each sample 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

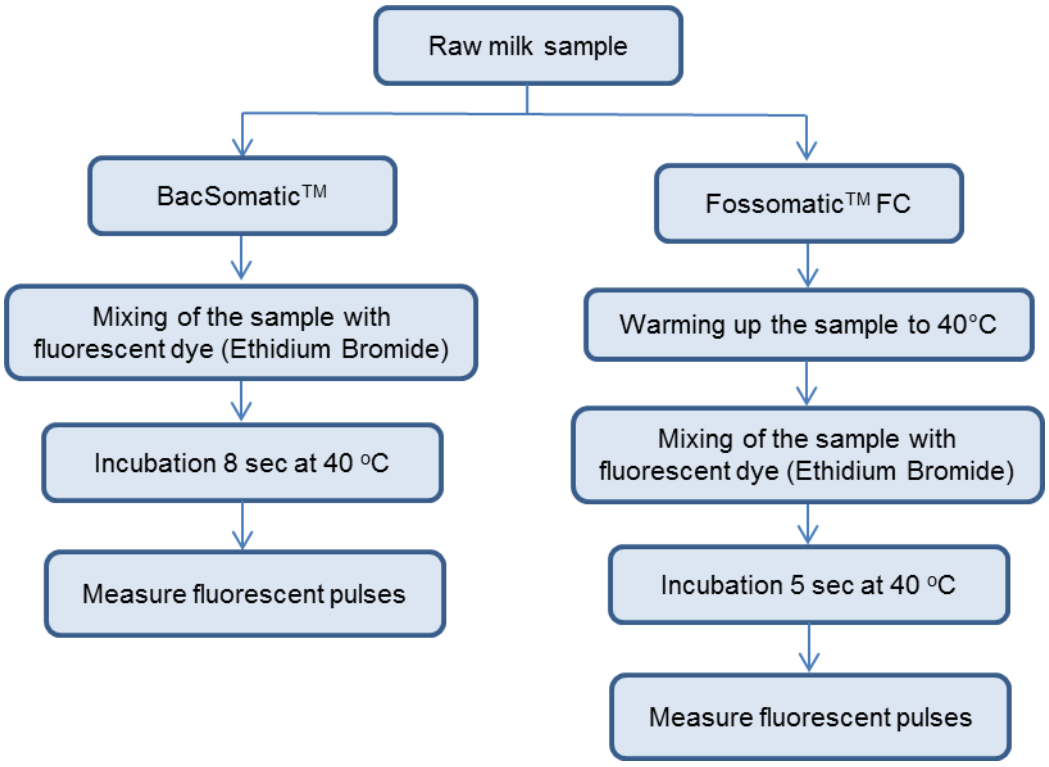
ISO 13366-1:2008 Milk - Enumeration of somatic cells - Part 1: Microscopic method (Reference method) (4).

1.5. Comparison instrument

Fossomatic™ FC with MicroVal certificate number 2015LR55.

1.6. Validation procedure

The measurement procedure for the direct comparison of BacSomatic™ and Fossomatic™ FC 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 (5). 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 BacSomatic™ 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 cells/mL – $2\ 000.10^3$ cells/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:

- BacSomatic™
- 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. Method 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 BacSomatic™ instrument

Cell count levels	Cell counts measured with BacSomatic™ (.10^3 cells/mL)
Low	181
Medium	563
High	1 583

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 BacSomatic™ in random order each 20 min during a working day with 20 checks in total. Routinely-available 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

Sample 12 from the low level and sample 11 from the medium level were excluded from the data analysis as they exceeded the limit of 3 times standard deviation (3*sd) from the mean of the group. Z-score was calculated and compared with the limit of 3*sd. These samples were measured in different sets and the calculated standard deviation of daily reproducibility ($s_{R,daily}$), standard deviation between checks (s_c) and standard deviation of means (s_x) were small and complying with the available requirements. It was concluded that the results were not caused by instability of the instrument. The stability was evaluated without sample 12 from the low level and sample 11 from the medium level. A summary of the 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 BacSomatic™ 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 (181.10^3 cells/mL)	7,4	4,0	0,0	7,4
Medium (563.10^3 cells/mL)	10,3	6,7	3,1	10,7
High (1583.10^3 cells /mL)	23,8	16,0	8,2	25,2

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 BacSomatic™ calculated per cell count level and acceptability values according to ISO 13366-2

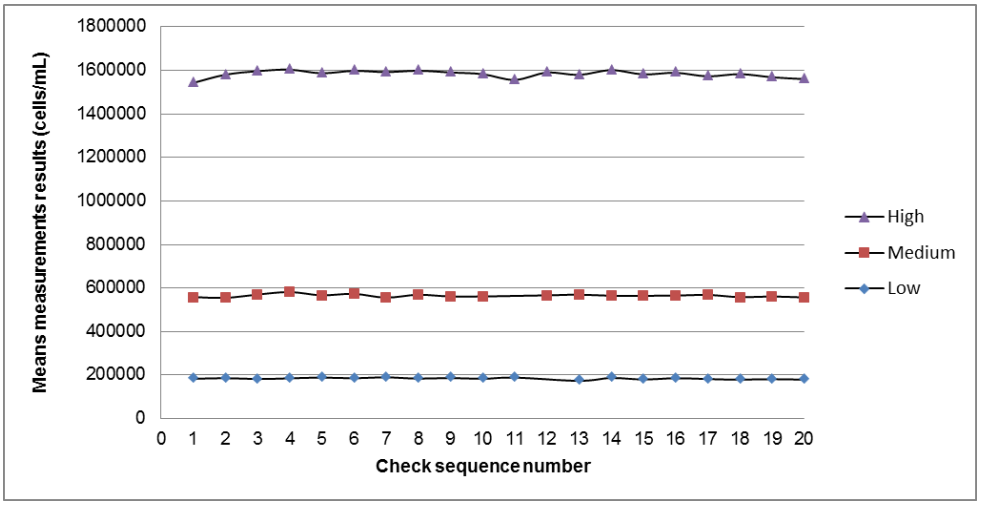
Cell count levels	s_r, calculated	s_r, acceptibility values according to ISO 13366-2*
	(.10^3 cells/mL)	
Low (181.10^3 cells/mL)	7,4	< 10,1
Medium (563.10^3 cells/mL)	10,3	< 22,5
High (1 583.10^3 cells /mL)	23,8	< 47,5
	(%)	
Low (181.10^3 cells/mL)	4	< 6
Medium (563.10^3 cells/mL)	2	< 4
High (1 583.10^3 cells /mL)	2	< 3

*the acceptability values presented in .10^3 cells/mL are calculated on the basis of the measured cell count levels and following 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) no official requirements exist.
The calculated standard deviation of daily reproducibility ($s_{R,daily}$) for each cell count level was small (Table 3) and, in fact, even complies with the requirements for standard deviation of repeatability.
The small standard deviation between checks (s_c) and standard deviation of means (s_x) 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. BacSomatic™ 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	$8{,}8.10^{2}$	19	$4{,}6.10^{1}$		
	Within group	$2{,}1.10^{3}$	37	$5{,}6.10^{1}$		
					0,82	1,88
	Total	$2{,}96.10^{3}$	56			
Medium	Between groups	$2{,}4.10^{3}$	19	$1{,}3.10^{2}$		
	Within group	$4{,}0.10^{3}$	37	$1{,}1.10^{2}$		
					1,17	1,88
	Total	$6{,}4.10^{3}$	56			
High	Between groups	$1{,}5.10^{4}$	19	$7{,}7.10^{2}$		
	Within group	$2{,}3.10^{4}$	40	$5{,}7.10^{2}$		
					1,35	1,85
	Total	$3{,}7.10^{4}$	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 BacSomatic™ functions stable throughout the working day and the stability complies with the requirements of the EURL MMP document (1) 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 BacSomatic™ was calculated based on the results of the above described stability experiment. For measurement protocol and calculations see clause 2.1.1.1.

Additionally the repeatability was calculated on 95 individual raw cow's milk samples and 225 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 %.

The results collected from the raw milk samples were also used for the evaluation of the intra-laboratory reproducibility ($R_{intra\text{-}lab}$) as described in clause 2.2.

Table 5. Raw cow's milk samples selected for estimation of the performance characteristics of the BacSomatic™

Cell count levels (.10^3 cells/mL)	Number of individual cow's milk samples	Cell count levels (.10^3 cells/mL)	Number of herd bulk cow's milk samples
50 - 200	23	50 - 200	89
201 - 400	14	201 - 400	88
401 - 1 000	21	401 - 1 000	32
1 001 - 2 000	37	1 001 - 2 000	16
Total number of samples	95	Total number of samples	225

All raw cow's milk samples were measured in duplicate (n = 2) on BacSomatic™. 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 BacSomatic™ instrument was calculated from the stability experiment (clause 2.1.1.2.) and the results and the acceptability values are given in Table 6.

Table 6. The repeatability (*r*) of the BacSomatic™ calculated from results obtained in the stability test 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 (181.10^3 cells/mL)	20,9	< 28,6
Medium (563.10^3 cells/mL)	29,1	< 63,7
High (1 583.10^3 cells /mL)	67,4	< 134,4
	(%)	
Low (181.10^3 cells/mL)	11	< 17
Medium (563.10^3 cells/mL)	6	< 11
High (1 583.10^3 cells /mL)	6	< 8

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

The calculated repeatability (*r*) for individual raw cow's milk and raw herd bulk cow's milk samples measured with BacSomatic™ instrument and the acceptability values are given Table 7, the number of samples per cell count level are in Table 5.

Table 7. The repeatability (*r*) of the BacSomatic™ calculated per cell count level for individual raw cow's milk 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	*r*, herd bulk cow's milk	*r*, acceptability values ISO 13366-2
		(.10^3 cells/mL)		
50 - 200	140	11,6	14,9	< 25,0
201 - 400	270	19,5	21,3	< 38,2
401 - 1 000	700	35,0	39,8	< 59,4
1 001 - 2 000	1500	51,3	91,9	< 126
		(%)		
50 - 200	140	8	11	< 17
201 - 400	270	8	8	< 14
401 - 1 000	700	5	5	< 8
1 001 - 2 000	1500	3	6	< 8

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

The calculated repeatability (*r*) for BacSomatic™ 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 BacSomatic™ complies with the requirement of EURL MMP document (1) 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.

Differences can 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 and used as a "high" samples for the evaluation of the carry-over of BacSomatic™. The carry-over was evaluated per cell count level separately. The cell count levels of the "high" samples are given in Table 8.

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

Cell count levels of the "high" samples	Theoretical ($.10^3$ cells/mL)	Measured ($.10^3$ cells/mL)
High 1	500	546
High 2	1 000	1 049
High 3	3 000	3 125

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

BacSomatic™ 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 (C) 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 carry-over from high to low ($C_{H/L}$) and carry-over from low to high sample ($C_{L/H}$) 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)	0,10	1,24
High 2 (ca.1 000.10^3 cells/mL	0,15	0,91
High 3 (ca.3 000.10^3 cells/mL	0,09	0,18

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

2.1.3.3. Conclusion

The carry-over effect with measurements on the BacSomatic™ complies with the requirements in EURL MMP document (1), C < 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 order of increasing cell count and in the second set 4 times in 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:

[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.

$$y = bx + a$$

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;

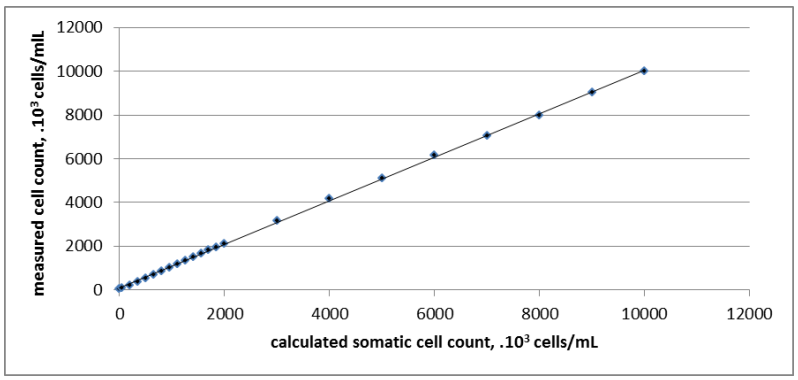
$M_{\min}$ is the numerical value of the lower measured value for the set of samples concerned.

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= 1,95 %. The results are pictured in Figure 2.

Figure 2. Linearity of BacSomatic™ in the testing range up to 10 000.10^3 cells/mL



The BacSomatic™ also appeared to be linear (r_C= 1,42 %) when more specifically examined in the instrument's 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 BacSomatic™ complies with the stated maximum limit value of r_C ≤ 2 % in the EURL document (1) 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. However, this is not the case for BacSomatic™ as described above.

The lower limit of quantification is the smallest amount of analyte 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 BacSomatic™. 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 BacSomatic™ 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 for determination of the lower limit of quantification of the BacSomatic™

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

The resulting lower limit of quantification is 7,4.10^3 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= 1,95 %. The results are pictured in Figure 2.

The upper limit of quantification of BacSomatic™ complies with the required >1 400.10^3 cells/mL in the EURL MMP requirement (1).

2.1.5.3. Conclusion

The lower limit of quantification of BacSomatic™ is 7.10^3 cells/mL.

The upper limit of quantification of BacSomatic™ is 10 000.10^3 cells/mL.

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

High contents of fat, protein and total bacterial count in the milk could interfere in somatic cell count measurements on the BacSomatic™. The influence of contents of fat, protein and total bacterial count 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, preserved raw cow's milk with 3,5, 4,5 and 5,5 % protein and preserved raw cow's milk spiked with yoghurt culture for total bacterial count of 5.10^4 cfu/mL, 2.10^5 cfu/mL, 8.10^5 cfu/mL and $1{,}5.10^6$ cfu/mL was adjusted at five cell count levels (ca. 200, 500, 800, 1 000 and 1 500.10^3 cells/mL).

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

Each sample was analysed four times with BacSomatic™.

The means of the replicate measurements per sample (n = 4) were calculated. The possible interference of high contents of fat, protein and total bacterial count on the 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 contents of fat, protein and total bacterial count with the somatic cell count. It was required that slopes are within the 95 % confidence limit interval of the calculated slope and intercept for samples with 3 % fat, 3,5 % protein and 5.10^4 cfu/mL or that there is an overlap between the 95% confidence limit intervals.

The relative linearity bias per fat, protein concentration and total bacterial 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.

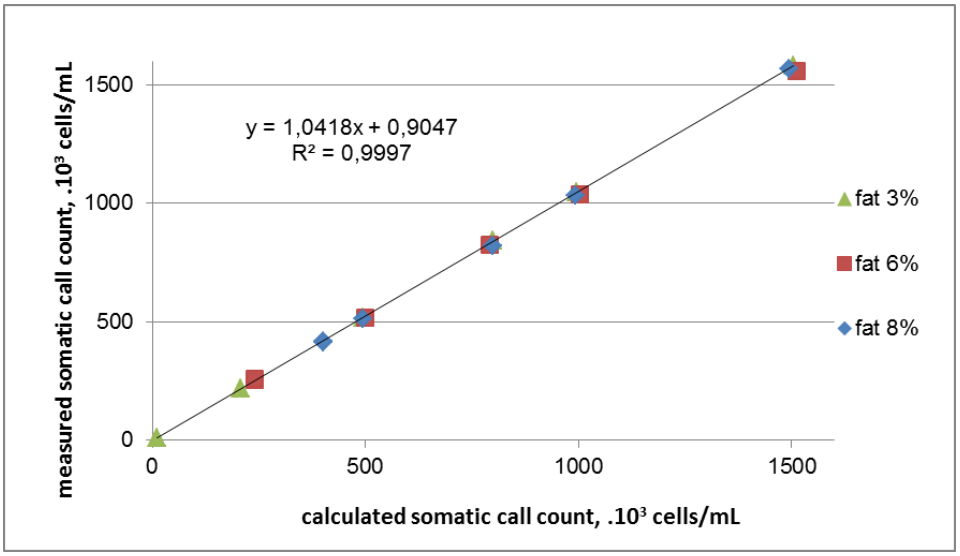
2.1.6.2. Results

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 BacSomatic™ on milk samples with different fat content and different somatic cell count levels are given in Table 11 and visualisation of all results and their linear regression analysis 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 BacSomatic™ on milk samples with different fat content and different somatic cell count levels

Fat	slope (*b*)			intercept (*a*) (.10^3 cells/mL)			r_C	s_{yx}
concentration	calculated	lowest 95%	highest 95%	calculated	lowest 95%	highest 95%	(%)	(%)
3%	1,0511	1,0450	1,0571	0,4167	-4,6649	5,4983	0,5	0,4
6%	1,0246	1,0161	1,0332	10,7786	3,5739	17,9832	0,7	0,4
8%	1,0522	1,0322	1,0723	-10,3732	-27,5521	6,8056	1,4	0,9

Figure 3. Linearity of the results obtained with BacSomatic™ 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 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 obtained with milk samples containing 3 % fat (Table 11). The slope obtained with 6 % fat (b = 1,0246) was slightly lower than the lowest limit of the 95 % confidence interval of the slope of milk with 3 % fat (b = 1,0450) and also slightly lower than the lowest limit of the 95 % confidence interval of the slope of milk with 8 % fat (b = 1,0322). The calculated 95 % confidence interval of the slope of milk with 6% fat was not overlapping with these calculated for milk with 3 % and 8 % fat. However the slope obtained with 8 % fat (b = 1,0522) was within the 95 % confidence interval obtained for the slope of milk with 3 % fat.

The 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 intercept obtained with milk samples containing 3 % fat. The intercepts obtained with 6 % and 8 % fat were outside the 95 % confidence intervals of 3 % fat. The 95 % confidence intervals of milk samples with 3 %, 6 % and 8 % fat were overlapping. To evaluate the effect of fat on the somatic cell count additional statistical analysis were performed to check normal distribution of the results with Shapiro test (6) and the standard deviations were compared with Bartlett's test (7). In the report it was pointed out 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 report is provided for MicroVal evaluation (8).

The calculated linearity ratio for each fat concentration was r_C < 2 % and the results obtained with BacSomatic™ on milk samples with increasing fat content and different somatic cell count levels appeared to be linear up to 1 500.10^3 cells/mL.

Accuracy was calculated as standard error (s_{yx}) and compared with s_{yx} ≤ 10 % required is ISO 8196-3. For all levels was s_{yx} ≤ 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 7), indicating no interference of the higher fat on the somatic cell count.

It was therefore concluded that milk fat content up to 8 % does not have a relevant influence on the somatic cell count result.

⇨ The somatic cell count results obtained with BacSomatic™ are not relevantly affected by an elevated fat content in the milk up to 8 %.

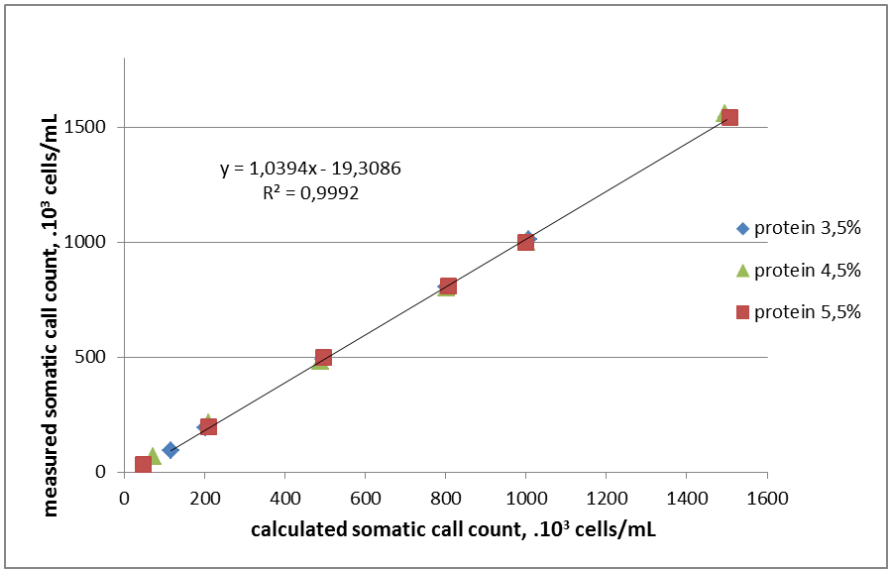
The calculated slope (*b*), intercept (*a*), 95 % confidence limit interval from linear regression analysis, linearity ratio (r_C) and standard error of accuracy (s_{yx}) on results obtained with BacSomatic™ on milk samples with different protein content and different somatic cell count levels are given 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 BacSomatic™ 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%	1,0395	1,0202	1,0588	-25,7879	-41,9285	-9,6473	1,4	1,2
4,5%	1,0365	0,9851	1,0878	-16,2893	-59,0883	26,5097	3,5	3,2
4,5%*	1,0445	0,9931	1,0959	-15,9321	-56,8068	24,9426	2,3	2,9
5,5%	1,0307	1,0109	1,0504	-17,8480	-34,3629	-1,3331	1,4	1,3

4,5%*- results without sample 5

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



The slope and intercept for each protein concentration level was calculated using linear regression. The slopes 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 obtained with milk

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

The calculated linearity ratio for 3,5 % and 5,5 % protein concentration was r_C < 2 %. The calculated linearity ratio for 4,5 % protein concentration was r_C > 2 %. This deviation from the linearity was caused by sample 5. By removing the samples from the analysis the linearity ratio (r_C = 2,3 %) was still slightly higher than the requirement, however the slope was within the 95 % confidence limit interval of milk with 3,5 % protein. It is concluded that no relevant influence of the protein content on the somatic cell count was observed.

Furthermore the accuracy was calculated as standard error (s_{yx}) and compared with s_{yx} ≤ 10 % required is ISO 8196-3. For all levels was s_{yx} ≤ 10 % (Table 12).

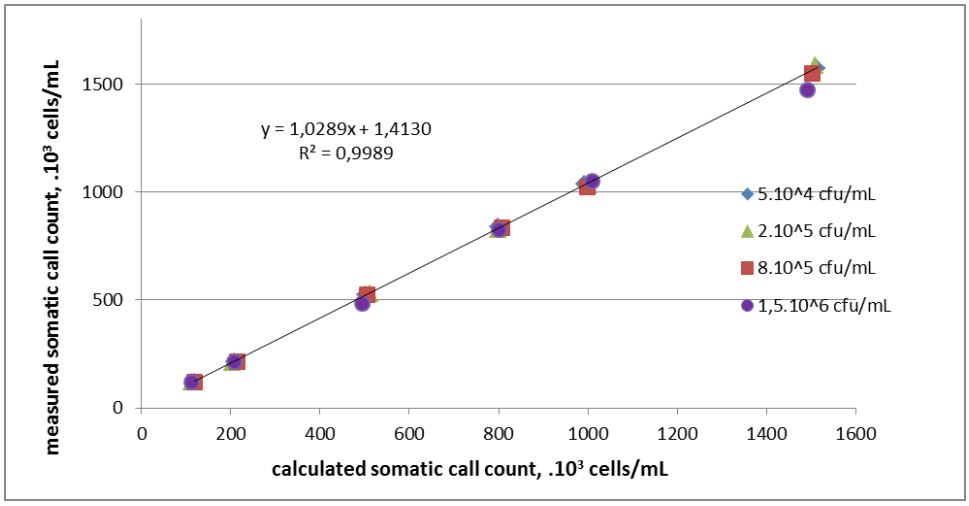
- ⇨ The somatic cell count results obtained with BacSomatic™ are not relevantly affected by an elevated protein content in the milk up to 5,5 %.

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 BacSomatic™ on milk samples with different total bacterial count and different somatic cell count levels are given in Table 13 and visualisation of the results is shown in Figure 5.

Table 13. 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 BacSomatic™ on milk samples with different total bacterial counts and different somatic cell count levels

Total bacterial count	slope (*b*)			intercept (*a*) (.10³ cells/mL)			r_C (%)	s_{yx} (%)
	calculated	lowest 95%	highest 95%	calculated	lowest 95%	highest 95%		
5.10⁴ cfu/mL	1,0410	1,0288	1,0532	1,3022	-8,9455	11,5499	0,9	0,7
2.10⁵ cfu/mL	1,0458	1,0258	1,0658	-5,1603	-21,9825	11,6618	1,4	1,1
8.10⁵ cfu/mL	1,0315	1,0201	1,0430	-1,3048	-10,9068	8,2972	0,8	0,7
1,5.10⁶ cfu/mL	0,9969	0,9366	1,0572	11,0737	-39,2397	61,3872	4,4	3,6

Figure 5. Linearity of the results obtained with the BacSomatic™ on milk samples with different total bacterial counts and different somatic cell count levels



The slope and intercept for each total bacterial count level was calculated using linear regression. The slopes and the 95 % confidence limit intervals obtained with milk samples with total bacterial count of 2.10^5 cfu/mL, 8.10^5 cfu/mL and $1,5.10^6$ cfu/mL were compared with the 95 % confidence limit interval of the slope obtained with milk samples containing 5.10^4 cfu/mL (Table 13). The slopes obtained with milk samples containing 2.10^5 cfu/mL, 8.10^5 cfu/mL were within the 95% confidence interval for the slope obtained with milk with total bacterial count 5.10^4 cfu/mL. For these samples the calculated linearity ratios were $r_C < 2$ %. The slope obtained with samples containing $1,5.10^6$ cfu/mL (b = 0,9969) was outside the 95 % confidence limit interval obtained for the slope of milk with 5.10^4 cfu/mL (b = 1,0410). The calculated 95 % confidence intervals of the both slopes were overlapping. The calculated linearity ratio for samples with high total bacterial count ($1,5.10^6$ cfu/mL) was $r_C > 2$ %. These deviations from linearity indicate that the somatic cell counts obtained with BacSomatic™ can be influenced by a total bacterial count higher than 8.10^5 cfu/mL.

The intercept and the 95 % confidence limit intervals obtained with milk samples with total bacterial count of 2.10^5 cfu/mL, 8.10^5 cfu/mL and $1,5.10^6$ cfu/mL were compared with the 95 % confidence limit interval of the intercept obtained with milk samples containing 5.10^4 cfu/mL. The intercepts obtained with milk samples containing total bacterial count of 2.10^5 cfu/mL, 8.10^5 cfu/mL and $1,5.10^6$ cfu/mL was within the 95% confidence interval for the intercept obtained with samples containing total bacterial count of 5.10^4 cfu/mL.

Accuracy was calculated as standard error (s_{yx}) and compared with $s_{yx} \leq 10$ % required is ISO 8196-3. For all levels was $s_{yx} \leq 10$ % (Table 13).

⇨ The somatic cell count results obtained with BacSomatic™ are not relevantly affected by a total bacterial count up to 8.10^5 cfu/mL. Higher concentrations of bacteria in the milk can influence the somatic cell count results.

To evaluate the effect of protein and total bacterial count on the somatic cell count additional statistical analysis were performed to check normal distribution of the results with Shapiro test (6) and the standard deviations were compared with Bartlett's test (7) (the analysis and results reported by AEOS (8). In the report it was indicated that that elevated fat, protein and somatic cell count could cause some noise in the results but do not influence the total bacterial count results. The report is provided for MicroVal evaluation.

2.1.6.3. Conclusions

No relevant influence of elevated milk contents of fat, protein and total bacterial count up to 8.10^5 cfu/mL was observed on the somatic cell count results obtained with the BacSomatic™. Higher concentrations of bacteria in the milk could influence the somatic cell count results.

2.2. Comparison of BacSomatic™ 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 BacSomatic™ was evaluated at different somatic cell count levels through comparison with the Fossomatic™ FC. $R_{intra\text{-}lab}$ was calculated with 95 individual raw cow's milk samples and 225 raw herd bulk cow's milk samples as shown in Table . From these samples 17 were with elevated fat content, > 5 %, and ware used in the analysis of the effect of fat content on the somatic cell counts, as described in clause 2.1.6.2.

The samples were measured in random order in duplicate with BacSomatic™ and were used for the calculation of the repeatability (r) of the instruments as described in clause 2.1.2. Single measurements of the same samples were performed with a Fossomatic™ FC instrument. 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 with the first result from duplicate measurement obtained with the BacSomatic™ and result obtained with the Fossomatic™ FC as:

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

where

x_1 = first result from duplicate measurement obtained with the BacSomatic™

x_2 = 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 models was visually inspected by plotting the results obtained with the BacSomatic™ 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 BacSomatic™ against Fossomatic™ FC was evaluated by linear regression analysis after natural logarithmic transformation of the results. The results were considered as equivalent when the calculated slope and intercept do not significantly differ 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 results obtained with BacSomatic™ and Fossomatic™ FC at different cell count levels were compared by calculating the intra-laboratory reproducibility ($R_{intra\text{-}lab}$). The intra-laboratory reproducibility results and the acceptability values are given in Table 14, the number of the samples per cell count level are given in Table 5.

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

Cell count levels (.10³ cells/mL)	Mean level samples (.10³ cells/mL)	$R_{intra\text{-}lab}$, individual cow's milk	$R_{intra\text{-}lab}$, herd bulk cow's milk	$R_{intra\text{-}lab}$, acceptability values ISO 13366-2
		(.10³ cells/mL)		
50 - 200	140	14,8	26,9	< 28,3
201 - 400	270	34,3	29,8	< 45,8
401 - 1 000	700	69,7	67,4	< 79,2
1 001 - 2 000	1500	91,2	156,6	< 169,8
		(%)		
50 - 200	140	10	19	< 20
201 - 400	270	13	11	< 17
401 - 1 000	700	9	9	< 11
1 001 - 2 000	1500	6	10	< 11

For each cell count level for individual cow's milk and herd bulk cow's milk samples the calculated intra-laboratory reproducibility of BacSomatic™ complies with the ISO 13366-2 acceptability values.

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

Figure 6. Relationship between BacSomatic™ and Fossomatic™ FC for individual raw cow's milk samples

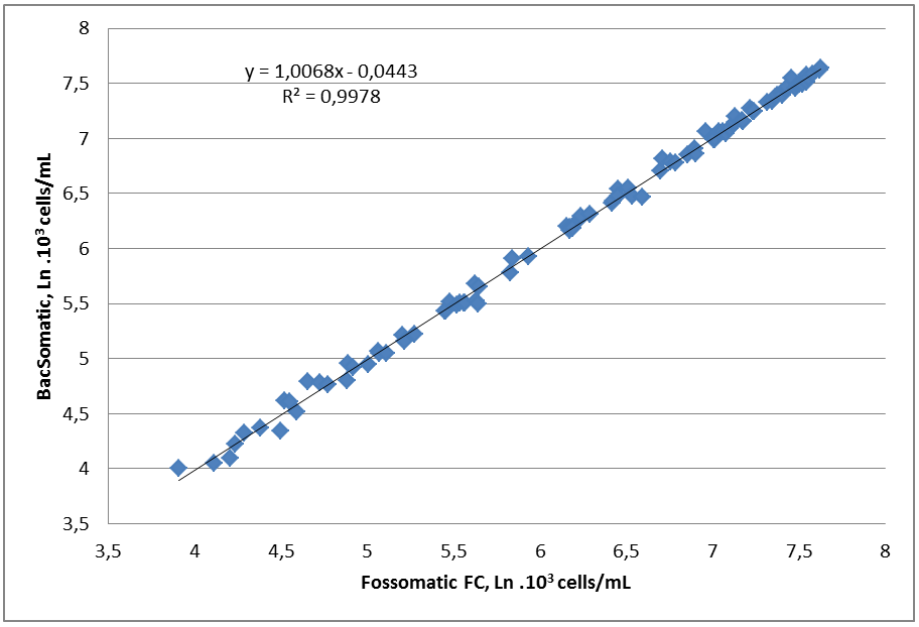


Figure 7a. Relationship BacSomatic™ and Fossomatic™ for raw herd bulk cow's milk samples in the range up to 2 000.10³ cell/mL

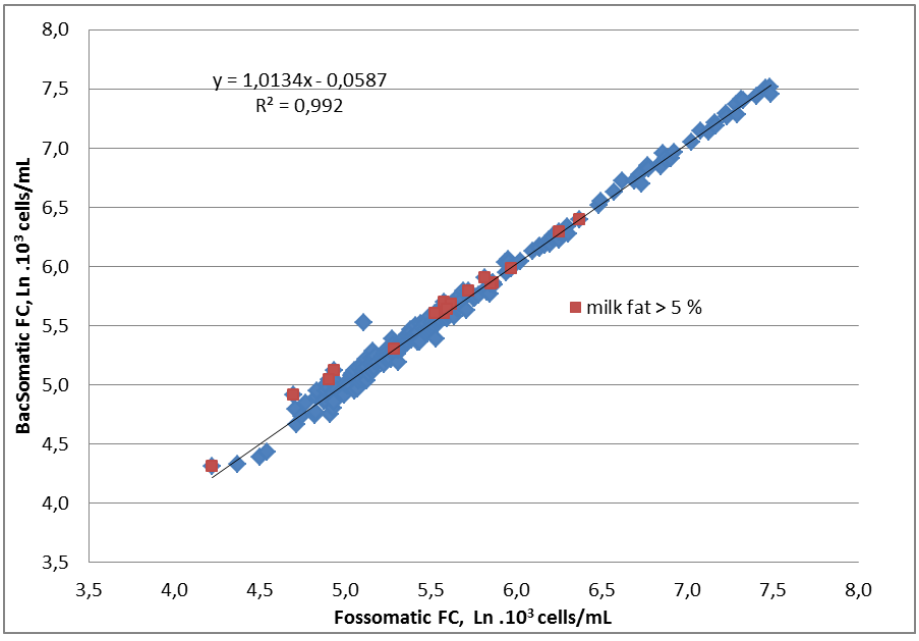
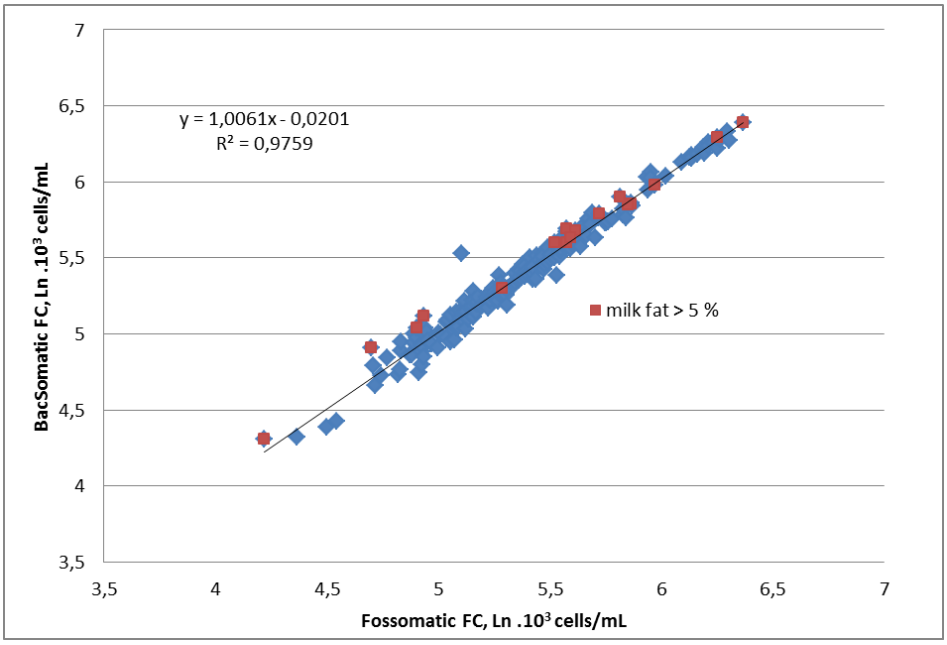


Figure 7b. Relationship BacSomatic™ and Fossomatic™ for raw herd bulk cow's milk samples in the range up to 600.10³ cell/mL



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 BacSomatic™ and Fossomatic™ FC on raw individual cow's and raw herd bulk cow's milk samples

	Coefficient	Lowest 95 %	Highest 95 %
Individual cow's milk samples, range up to 2 000.10³ cell/mL			
slope	1,0068	0,9970	1,0166
intercept	-0,0443	-0,1063	0,0177
Herd bulk cow's milk samples, range up to 2 000.10³ cell/mL			
slope	1,0134	1,0014	1,0250
intercept	-0,0587	-0,1264	0,0090
Herd bulk cow's milk samples, range up to 600.10³ cell/mL			
slope	1,0061	0,9836	1,0285
intercept	-0,0201	-0,1415	0,1013

The 95 % confidence limit interval of the slope and the intercept for individual cow's milk samples included respectively 1 and 0, meaning that the relationship between the results obtained with BacSomatic™ and Fossomatic™ FC were statistically identical at threshold $p < 0,05$. The calculated standard error of the results was small, s_{yx} = 0,05 Ln.10³ cells/mL

The 95 % confidence limit interval of the intercept for herd bulk cow's milk samples in the evaluated range up to 2 000.10³ cells/mL included 0, however the theoretical slope = 1 was just outside the 95 % confidence limit interval of the calculated slope. This deviation was caused by the samples with somatic cell count > 600.10³ cells/mL. These samples were artificially prepared by spiking herd bulk

milk with milk leucocyte suspension. The regression analysis was performed on the natural herd bulk cow's milk samples up to 600.10^3 cells/mL. The 95 % confidence limit interval of the slope and the intercept included respectively 1 and 0, meaning that the relationship between the results obtained with BacSomatic™ and Fossomatic™ FC were statistically identical at threshold $p < 0,05$. The calculated standard error of the results was small, s_{yx} = 0,06 Ln.10^3 cells/mL.

The small standard error (s_{yx}) and the not significant deviation of the regression line from the identity function demonstrated a close correlation between the results obtained with both instruments and indicate that BacSomatic™ and Fossomatic™ FC can be considered equivalent.

Note: The raw individual cow's and herd bulk milk samples used in the comparative evaluation of BacSomatic™ were measured simultaneously for total bacterial and somatic cell counts (combi) mode of BacSomatic™. The same samples were measured with BactoScan™ FC/FC+ and Fossomatic™ FC, both granted with MicroVal certificates. The simultaneous determination of total bacterial and somatic cell counts demonstrated the performance of BacSomatic™ in its routine modus. The total bacterial count results were used for the MicroVal validation of BacSomatic™ in terms of total bacterial counting (MicroVal Project 2016LR65).

2.2.3.Conclusion

The somatic cell count results obtained with BacSomatic™ are equivalent to those obtained with Fossomatic™ FC for both individual raw cow's milk and raw herd bulk cow's milk samples.

3. Conclusions

The method comparison study

BacSomatic™ performance characteristics determined according to ISO 8196-3 and ISO 13366-2 are:

- BacSomatic™ 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) 6 % *(ISO 13366-2: ≤ 8 %)*
- Carry-over (C) per cell count level (*ISO 13366-2: for each cell count level C < 2 %)*
  - Low (ca. 500.10^3 cells/mL) $C_{H/L}$ = 0,10 %; $C_{L/H}$ = 1,24 %
  - Medium (ca. 1 000.10^3 cells/mL) $C_{H/L}$ = 0,15 %; $C_{L/H}$ = 0,91 %
  - High (ca. 3 000.10^3 cells/mL) $C_{H/L}$ = 0,09 %; $C_{L/H}$ = 0,18 %
- Linearity (r_C): 1,4 % *(ISO 13366-2: r_C ≤ 2 %)*
- Lower limit of quantification (L_Q): 7.10^3 cells/mL
- Upper limit of quantification: 10 000.10^3 cells/mL
- Evaluation of factors that possibly interfere with somatic cell count results:

High fat (up to 8 %), protein (up to 5,5 %) content and total bacterial count (up to 8.10^5 cfu/mL) of the milk did not relevantly influence the somatic cell count results determined with the BacSomatic™.

The comparison of BacSomatic™ and Fossomatic™ FC

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

- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in individual raw cow's milk:
  - Cell level 50 - 200.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 20 %)*
  - Cell level 201 - 400.10^3 cells/mL 13 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401 – 1 000.10^3 cells/mL 10 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 1 001 - 2 000.10^3 cells/mL 6 % *(ISO 13366-2: ≤ 11 %)*
- Intra-laboratory reproducibility ($R_{intra\text{-}lab}$) per cell count level in 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 11 % *(ISO 13366-2: ≤ 17 %)*
  - Cell level 401 – 600.10^3 cells/mL 6 % *(ISO 13366-2: ≤ 11 %)*
  - Cell level 601 - 2 000.10^3 cells/mL 11 % *(ISO 13366-2: ≤ 11 %)*
- The 95 % confidence limit interval of the slope and the intercept of the results obtained from individual raw cow's milk and the natural raw herd bulk cow's milk samples included respectively 1 and 0
- Standard error (s_{yx}) of the results is small:
  - for individual raw cow's milk s_{yx}= 0,05 Ln.10^3 cells/mL
  - for raw herd bulk cow's milk s_{yx}= 0,06 Ln.10^3 cells/mL

It is concluded that the results obtained with BacSomatic™ and Fossomatic™ FC are equivalent for all cell count levels.

Final conclusion of the validation study

The final conclusion of the validation study is:

All results obtained during the method comparison study of BacSomatic™ comply with the criteria of the EURL MMP document. The direct comparison of results from BacSomatic™ and Fossomatic™ FC (MicroVal certificate 2015LR55) revealed equivalence in terms of enumeration of somatic cells and do comply with the criteria of the EURL MMP document.

4. References

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