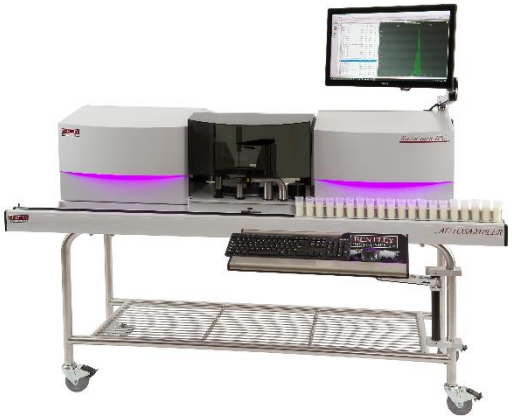
ARTICLE

MICROVAL VALIDATION OF THE BACTOCOUNT™ IBC3.0 FOR THE ENUMERATION OF SOMATIC CELLS AND TOTAL BACTERIAL COUNT IN RAW COW MILK (2021LR97)

The BactoCount™ IBC3.0 is an automatic instrument that uses flow cytometry for the rapid, accurate and highly reliable enumeration of individual bacteria (TBC) and somatic cells (SCC) in raw milk. It was developed by the BENTLEY instruments company (US) and distributed in Western Europe by Bentley Instruments SARL (www.bentleyinstruments.eu). The BactoCount™ IBC3.0 was developed for the Central Milk Testing laboratories and large dairy plants for milk payment.

Total bacterial count and somatic cell count can be performed combined or individually. **In the frame of the validation, SCC and TBC were tested separately.**

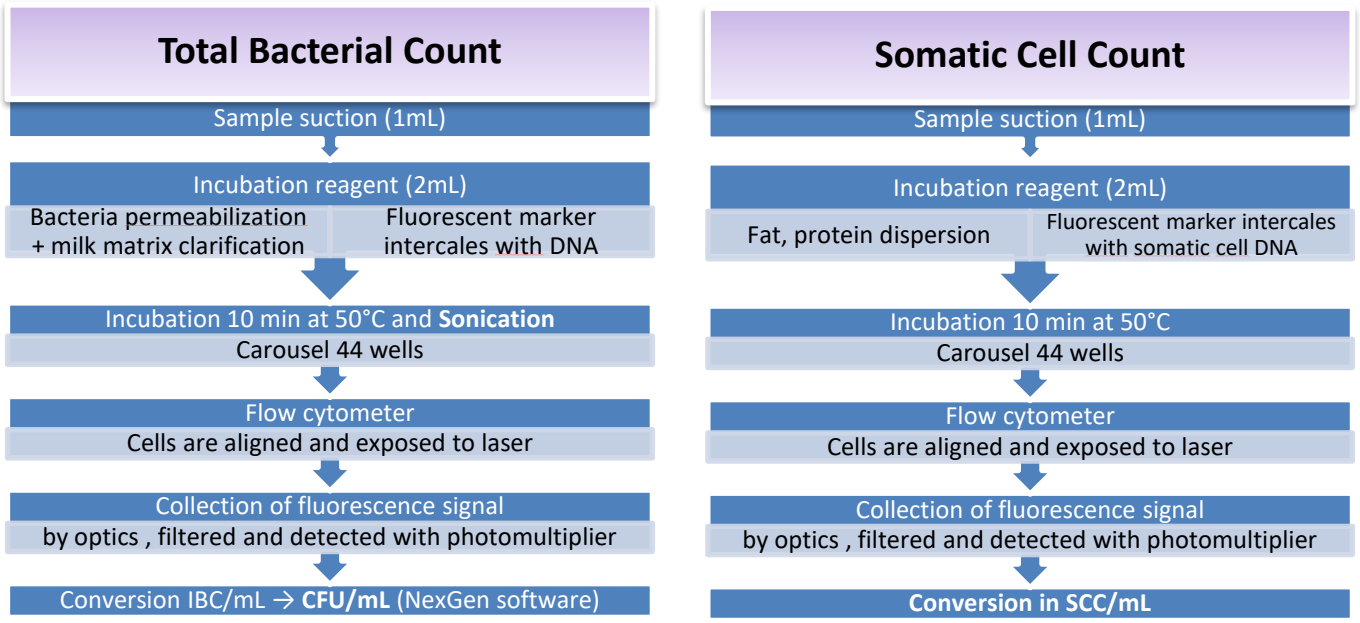
ACTALIA Cecalait was chosen as expert laboratory to conduct the MicroVal validation study on this device (Validation number 2021LR97). This document is a summary of the results obtained during the validation study.



PRINCIPLE OF THE ALTERNATIVE METHOD

The alternative method protocol is based on **flow cytometry principle**, where the DNA contents in cells (somatic cells or bacteria) are stained with a fluorescent marker, then detected through fluorescence signal. This signal is finally converted into universal unit thanks to the Bentley's software, NexGen.

The BactoCount™ IBC3.0 can analyze up to 200 samples/hour by using a **44-well carousel** and a **rack sampler**.



ARTICLE

PRINCIPLE AND CONDITIONS OF THE VALIDATION

The validation study was conducted in accordance with the ISO 16140-2, ISO 16297, ISO 21187 and EU-RL MMP criteria for TBC and with the ISO 16140-2, ISO 8196-3, ISO 13366-2 and EU-RL MMP criteria for SCC.

The method comparison study conducted for the MicroVal Validation was divided in the two following main parts:

- **The evaluation of the preliminary performances** of the device (stability, carry-over effect between samples, linearity and limit of quantification);

- **The evaluation of the repeatability and the accuracy** of the instrument.

The performance characteristics of the alternative method have been evaluated using calibrated milk samples:

- For TBC, raw cow milk was spiked with one strain of *Lactococcus lactis* to obtain specific concentrations of total bacteria. Each milk sample was used during the day and was not stored. The milk samples were placed between 0 and +4°C before the measurement;
- For SCC, raw cow milk was skimmed and microfiltered to obtain 2 suspensions: one with higher (concentrate) and one with lower concentration (filtrate) of somatic cells (according to ISO 13366-2). A range of samples was prepared to have specific concentration of somatic cells. Each milk sample was used during the day and was not stored. The milk samples were placed in a water bath at 40±2°C for 20 minutes before the SCC measurement.

The repeatability and the accuracy of the instrument were evaluated on herd raw cow milk samples for TBC and by using individual and herd raw cow milk samples for SCC.

Concerning accuracy testing, the results obtained with the alternative method were compared to the results obtained with Bentley's devices already validated:

- BactoCount™ IBC2.0 for TBC (MicroVal certified; certificate n°2013 LR 44);
- SomaCount™ FC for SCC (ICAR certified according ISO 8196-3; certificate n°2020/7).

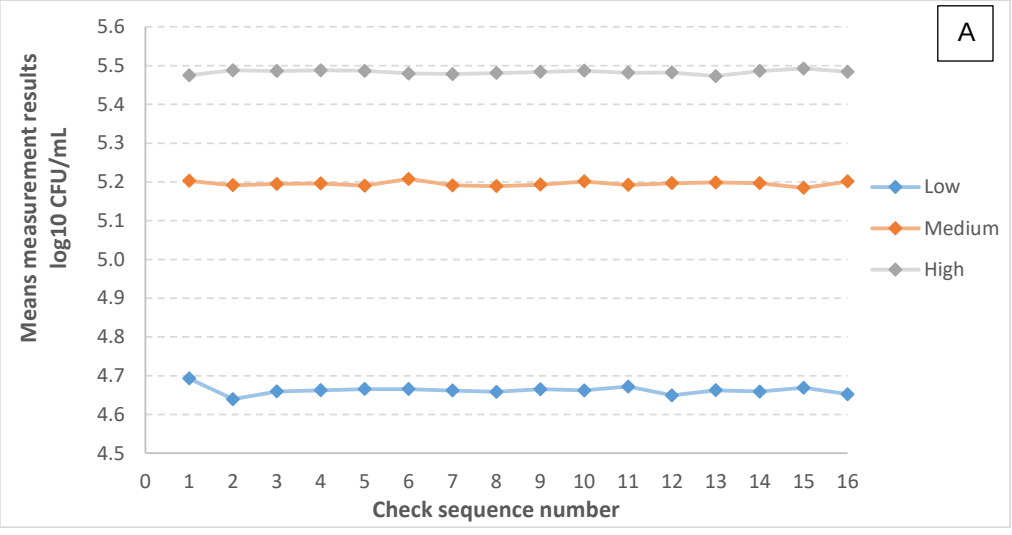
1. EVALUATION OF THE STABILITY, CARRY-OVER EFFECT BETWEEN SAMPLES, LINEARITY AND LIMIT OF QUANTIFICATION

1.1 – Stability

The stability of the alternative method was verified by mimicking routine testing circumstances throughout a working day. To evaluate the stability of the instrument, calibrated milk samples were analyzed every 15-20 minutes during a day of working. The concentration of the calibrated samples used in the stability study are listed in **Table 1**.

Table 1: TBC and SCC levels of the samples used for the stability evaluation

Level	TBC (Log_{10} CFU/ml)	SCC ($x10^3$/ml)
Low	4.7	73
Medium 1	5.2	492
Medium 2	-	996
High	5.5	1439



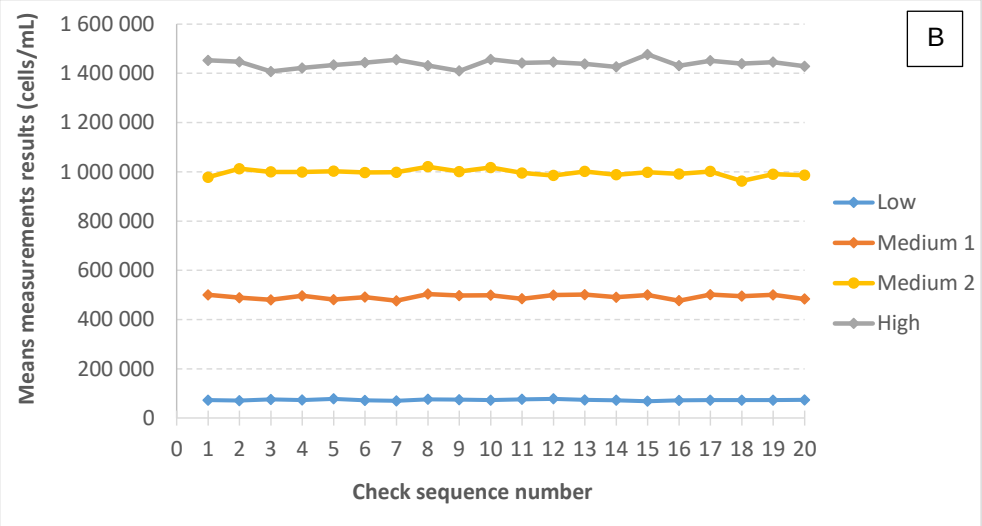


Figure 1: BactoCount™ IBC3.0 stability for TBC (A) and SCC (B) throughout the working day

For each level, the standard deviation of repeatability (*Sr*) and the standard deviation of daily reproducibility ($S_{R,daily}$) were determined (**Table 2**). All results were lower than the acceptability limits defined in the ISO 16297 and the EU-RL MMP document for the TBC and the limits defined in the ISO 8196-3 for the SCC.

Table 2: BactoCount™ IBC3.0 stability results for TBC and SCC

		S_r	$S_{R,daily}$
TBC (Log_{10} CFU/ml)	**Low level**	0.03	0.03
	Medium level	0.01	0.01
	High level	0.01	0.01
SCC (x 10^3 cells/ml)	**Low level**	4.8	4.8
	Medium 1 level	11.9	13.2
	Medium 2 level	18.9	20.3
	High level	22.3	24.4

The results obtained during the evaluation of the stability suggest that the BactoCount™ IBC3.0 is stable in the working day for TBC and SCC in raw cow milk.

1.2 – Carry-over effect between samples

Strong differences in TBC or SCC levels between two successively analyzed samples may influence the result of the second one. Carry-over effect may occur in analytical systems with continuous flow systems. It is linked to the transfer of a certain portion of sample to the next or further samples.

For evaluation of the carry-over effect of the instrument, calibrated milk samples were prepared: 4 levels for TBC (near to 50 / 150 / 300 and 1500x10^3 CFU/mL) and 3 levels for SCC (near to 500 / 1000 and 1500x10^3 cells/mL). These milks were analyzed alternatively with blank milk samples. The carry-over effect determined reflects the contamination of the high milk on the blank milk measured just after. The carry-over effect was evaluated and calculated according to the ISO 8196-3 (**Table 3**). The calculated carry-over effect between samples was lower than the limit of 1% for TBC (each level and total samples) and lower than the limit of 2% for SCC (each level and total samples).

Table 3: Carry-over effect of high milk samples (3 or 4 levels of concentration) on blank milk samples determined for TBC and SCC

	CO effect (%)	
	TBC	**SCC**
Level 1	0.91 %	0.69%
Level 2	0.74 %	0.59%
Level 3	0.65 %	0.44%
Level 4	0.45 %	-
Total	**0.69 %**	**0.53%**

Results of carry-over between samples evaluation fulfill the requirements of the ISO 16297 for TBC and the ISO 8196-3 for SCC.

1.3 – Linearity and limits of quantification

For TBC, the linearity of the BactoCount™ IBC3.0 was evaluated in the range from $5x10^2$ and $5x10^6$ CFU/mL with calibrated milk samples (**Figure 2**).

For SCC, the linearity was evaluated from 0 to 2 500 x 10^3 cells/mL with calibrated milk samples (**Figure 2**).

With a calculated ratio (r_L) of **3.2%** for TBC and a ratio (r_C) determined at **0.76%** for the SCC, the instrument fulfills to the limits defined in the ISO 16297 (**< 5%**) and ISO 8196-3 (**< 2%**) respectively.

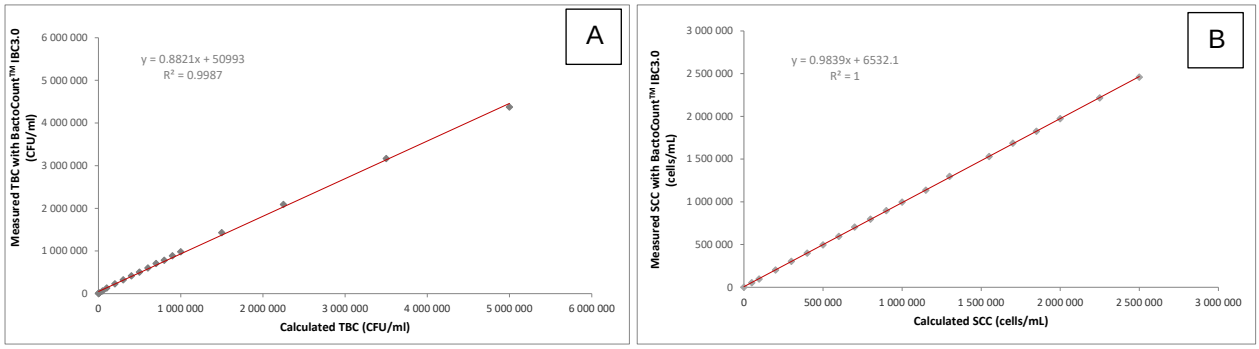


Figure 2: Linearity of the BactoCount™ IBC3.0 for measurement of TBC (A) and SCC (B)

For TBC, the lower limit of quantification was defined at 5 000 CFU/mL and the measurements are linear up to $5x10^6$ CFU/mL.

For SCC, the lower limit of quantification was defined at 10 000 cells/mL and the measurements are linear up to $2500x10^3$ cells/mL.

2. EVALUATION OF THE REPEATABILITY

The repeatability of the instrument was evaluated by analysing:

- For TBC: **250 raw herd bulk cow milk samples** representative for different total bacterial count levels;
- For SCC: **135 individual raw cow milk samples** and **67 raw herd bulk cow milk samples** representative for different somatic cell count levels.

For SCC, the instrument was pre-calibrated using ACTALIA Cecalait's somatic cells standard reference materials.

All samples were measured in duplicate (n=2) with the BactoCount™ IBC3.0. For interpretation of results, the samples were sorted in different ranges of concentrations in bacteria and somatic cells (ranges defined in the ISO 16297 and the ISO 13366-2 respectively). The standard deviation of repeatability (Sr) or the repeatability (*r*) was calculated for each count level (**Table 4**).

Table 4: BactoCount™ IBC3.0 - Repeatability criteria for total bacterial count and somatic cells count

		Number of samples	Mean level samples	S_r / *r*	Acceptability values (ISO 16297 or ISO 13366-2)
TBC (Log_{10} CFU/ml)	**<4.3**	85	4.0	**0.07**	0.12
	≥4.3	165	4.9	**0.05**	0.09
SCC (x 10^3 cells/ml)	**0-150**	143	60	**15**	25
	150-300	36	205	**22**	42
	300-450	11	355	**29**	50
	450-750	8	571	**28**	63
	750-1500	4	960	**29**	126
	All	202	140	**18**	-

Repeatability of the BactoCount™ IBC3.0 for the TBC and SCC respectively complies with the requirement of ISO 16297, EU-RL MMP document and ISO 13366-2.

3. EVALUATION OF THE ACCURACY

The accuracy of the instrument was evaluated by using:

- For TBC: **246 unpreserved raw herd bulk cow milk samples**;
- For SCC: **134 individual raw cow milk samples** preserved with bronopol and **66 unpreserved raw herd bulk cow milk samples**.

All samples were measured in duplicate (n=2) with the alternative method and the anchoring method (BactoCount™ IBC2.0 for TBC / SomaCount™ FC for SCC). Results obtained with the 2 methods were plotted (**Figure 3** and **Figure 4**) and the residual standard deviation ($s_{y,x}$) was calculated.

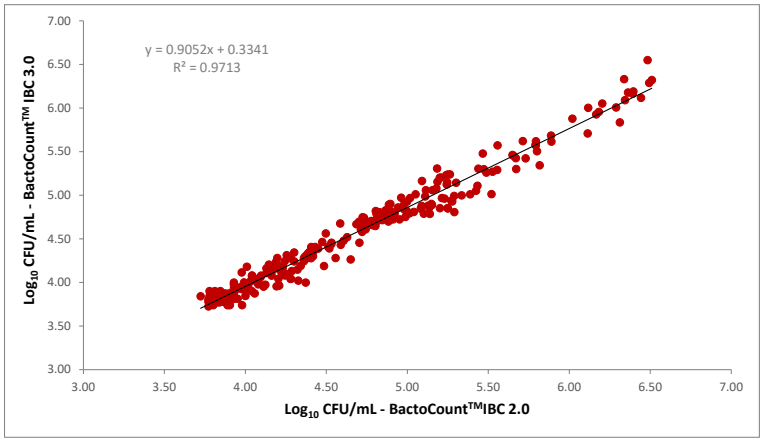


Figure 3: Relation between the BactoCount™ IBC3.0 and the BactoCount™ IBC2.0

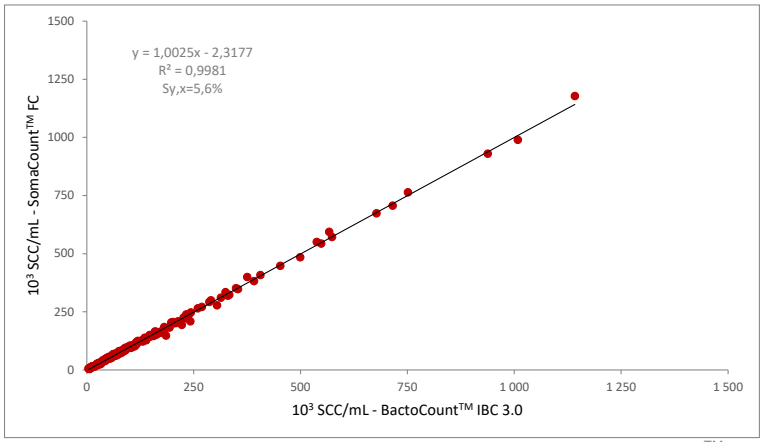


Figure 4: Relation between the BactoCount™ IBC3.0 and the SomaCount™ FC

For TBC, the residual standard deviation determined was **$s_{y,x}$ = 0.11** $\log_{10}$ CFU/ml and complies with the limit of **0.40** $\log_{10}$ CFU/mL defined in the ISO 16297 and EU-RL MMP document. The residual standard deviation is also within the ISO 16297 reproducibility limit (**<0.16 log**).

For SCC, the residual standard deviation determined for SCC measurements for each range of concentrations and for the total of samples (**$s_{y,x}$ = 5.6%**) is lower than the limit defined in the ISO 8196-3 (**8 %**).

The accuracy of the BactoCount™ IBC3.0 for TBC and SCC fulfill to the ISO 16297, the ISO 8196-3 and the EU-RL MMP documents.

4. CONCLUSION

ACTALIA Cecalait conducted a complete MicroVal Validation Study of the BactoCount™ IBC3.0 for the separately measurements of TBC and SCC in raw cow milk. This study was performed according to the general ISO 16140-2 requirements and the more specific ISO 16297, ISO 21187, ISO 8196-3 and ISO 13366-2 depending on the target.

For all the tested conditions, the instrument was considered as **stable** in the working day for TBC and for SCC and the **carry-over effect between samples** measured was lower than the requirements. The **linearity** of the measurements was checked on the usual measurement range and the **lower limits of quantification** were determined. The **repeatability** of the device also complies with the requirements.

For the TBC, the comparison with the anchoring method (BactoCount™ IBC2.0 - MicroVal certified; certificate n°2013 LR 44) revealed equivalence in terms of enumeration of bacteria and do comply with the criteria of the EU-RL MMP document.

For the SCC, the comparison with the anchoring method (SomaCount ™ FC - ICAR certified according to ISO 8196-3; certificate n°2020/7) revealed equivalence in terms of enumeration of somatic cells and do comply with the criteria of the EU-RL MMP document.

Finally, taking into account all results of this validation study, the BactoCount™ IBC3.0 complies with the standards requirements for Total Bacterial Count and for Somatic Cell Count in raw cow milk.

On the basis of this evaluation study, the instrument has been validated by MicroVal for TBC and SCC in raw cow milk.

Standards used for this evaluation:

- *For TBC:* *- ISO 16140-2*
  - ISO 16297
  - ISO 21187
  - EU-RL MMP criteria

- *For SCC:* *- ISO 16140-2*
  - ISO 8196-3
  - ISO 13366-2
  - EU-RL MMP criteria

According to the MicroVal validation report of the BactoCount™ IBC3.0 – Delphine LAROSE
MicroVal Validation number: 2021LR97