CECALAIT-S NEWSLETTER

ACTALIA
CECALAIT

1st quarter 2024, No. 127

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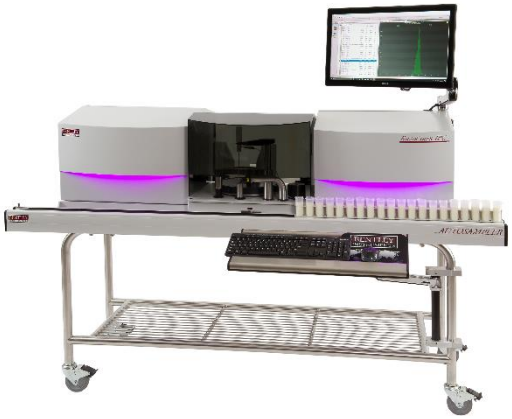
ARTICLE

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.**

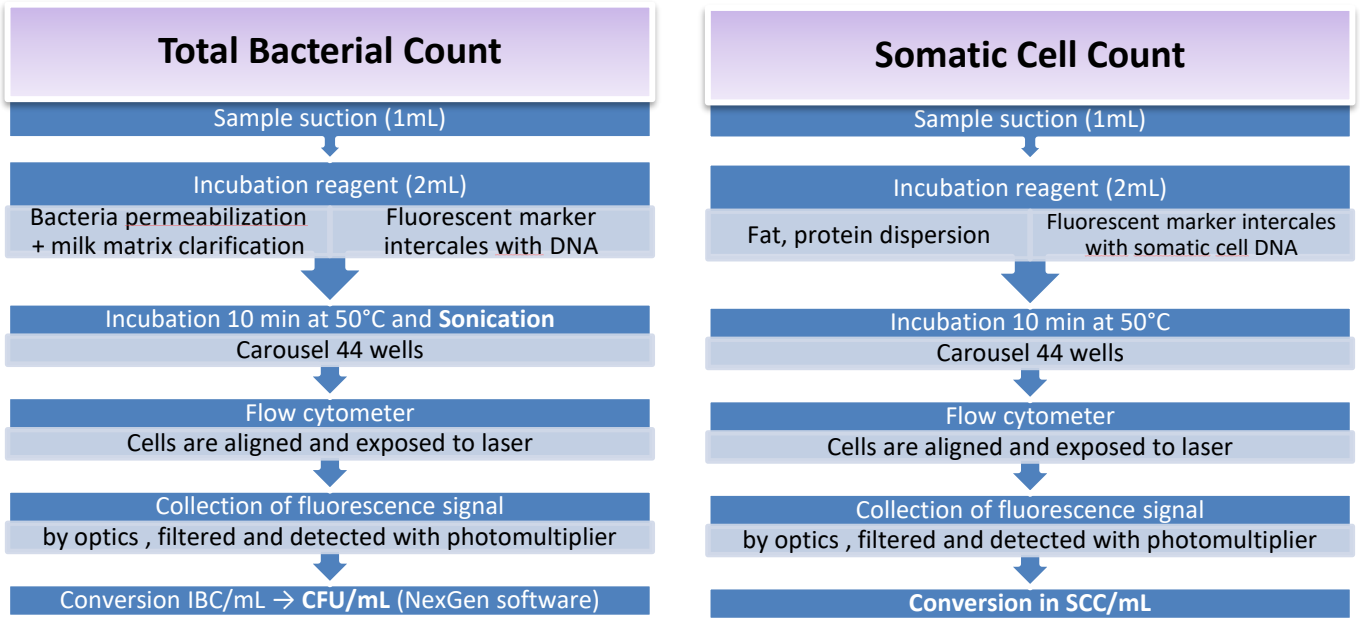
ACTALIA Cecalait was chosen as expert laboratory to conduct the MicroVal validation study on this device (Validation number 2021LR97). This document is a summary of the results obtained during the validation study.



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**.



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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).

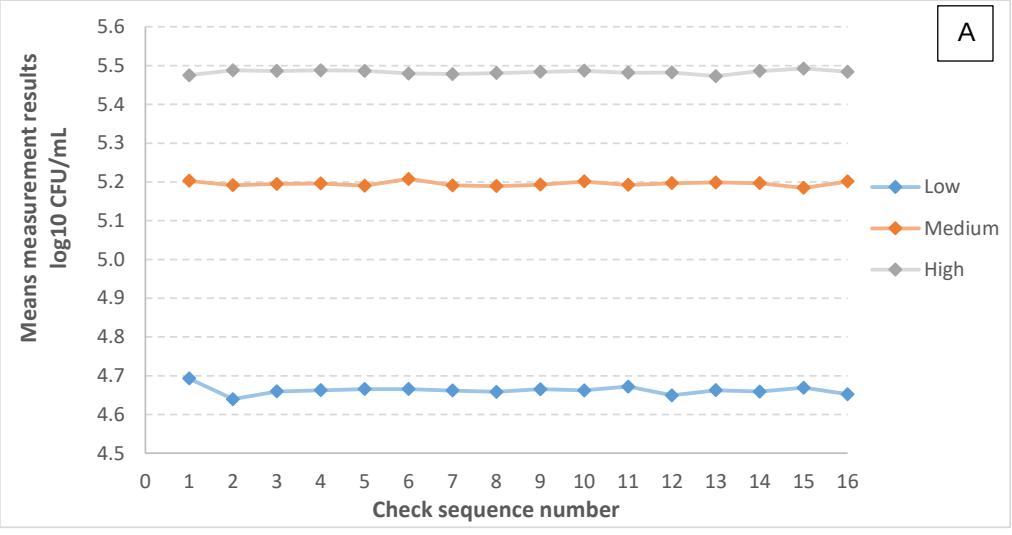
1. EVALUATION OF THE STABILITY, CARRY-OVER EFFECT BETWEEN SAMPLES, LINEARITY AND LIMIT OF QUANTIFICATION

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



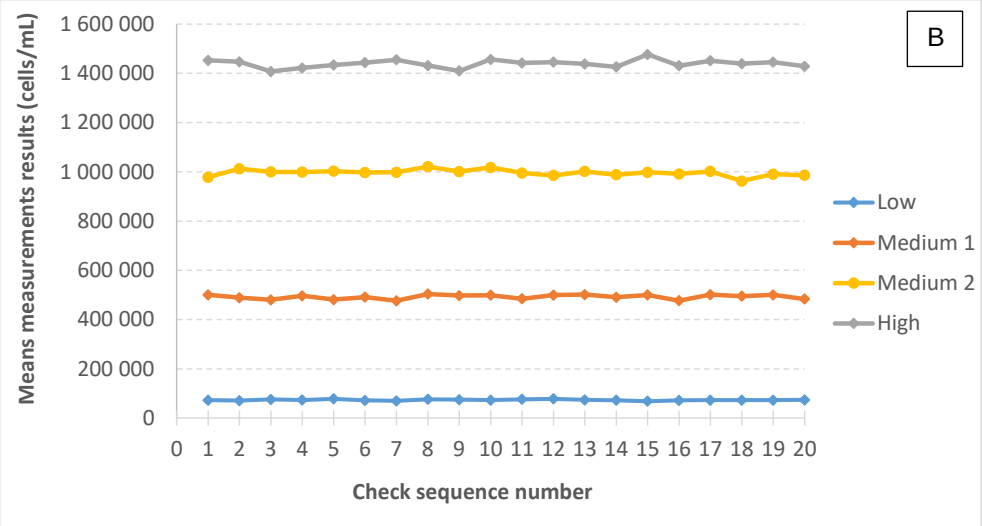


Figure 1: BactoCount™ IBC3.0 stability for TBC (A) and SCC (B) throughout the working day

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.

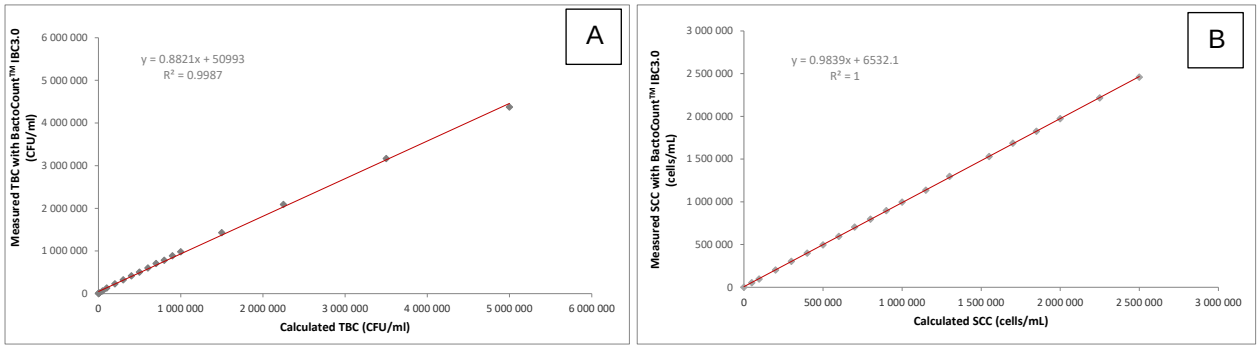


Figure 2: Linearity of the BactoCount™ IBC3.0 for measurement of TBC (A) and SCC (B)

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.

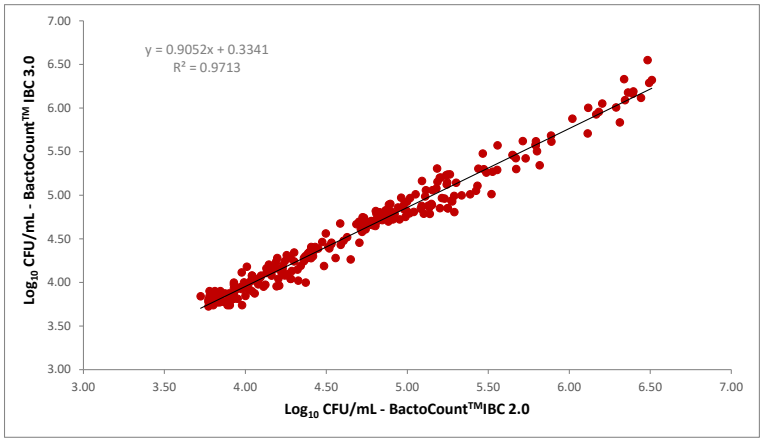


Figure 3: Relation between the BactoCount™ IBC3.0 and the BactoCount™ IBC2.0

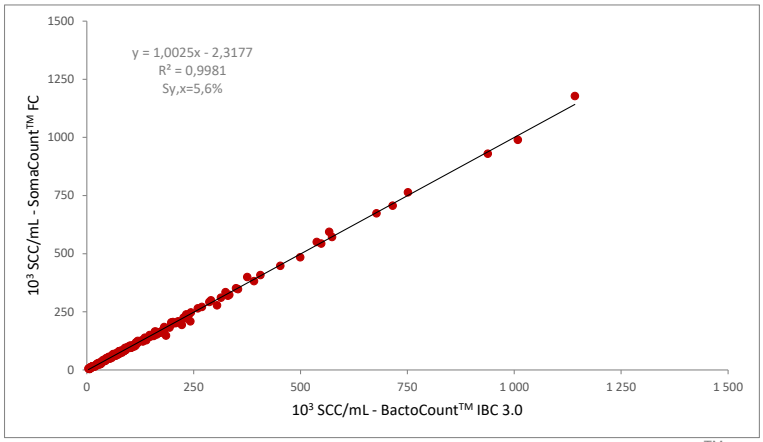


Figure 4: Relation between the BactoCount™ IBC3.0 and the SomaCount™ FC

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

ARTICLE

MICROVAL VALIDATION OF THE BACTOCOUNT™ IBCm3.0 FOR THE ENUMERATION OF SOMATIC CELLS AND TOTAL BACTERIAL COUNT IN RAW COW MILK (2021LR98)

The BactoCount™ IBCm 3.0 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™ IBCm 3.0 was developed to allow the dairy plants to check accurately and rapidly the hygienic quality of their incoming raw milk before unloading and processing it.

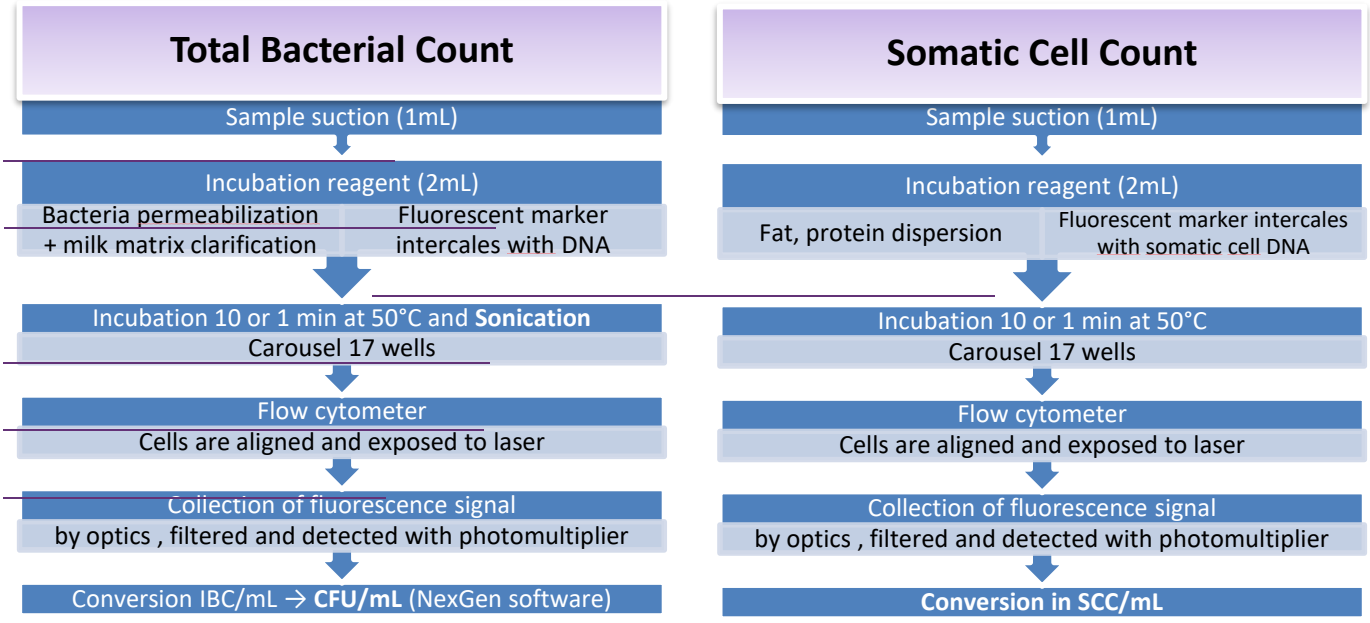
Total bacterial count and somatic cell count can be performed combined or individually. **In the frame of this validation, SCC and TBC were tested separately.**

ACTALIA Cecalait was chosen as expert laboratory to conduct the MicroVal validation study on this device (Validation number 2021LR98). 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™ IBCm3.0 can analyze **up to 50 samples/hour** by using a **17-well carousel and a conveyor system.** Moreover, it offers 2 programs for analyze of TBC and SCC: the **10min test** (10' test) and the **2min test** (2' test).These two programs were tested in the frame of the Validation study.



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 limits 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).

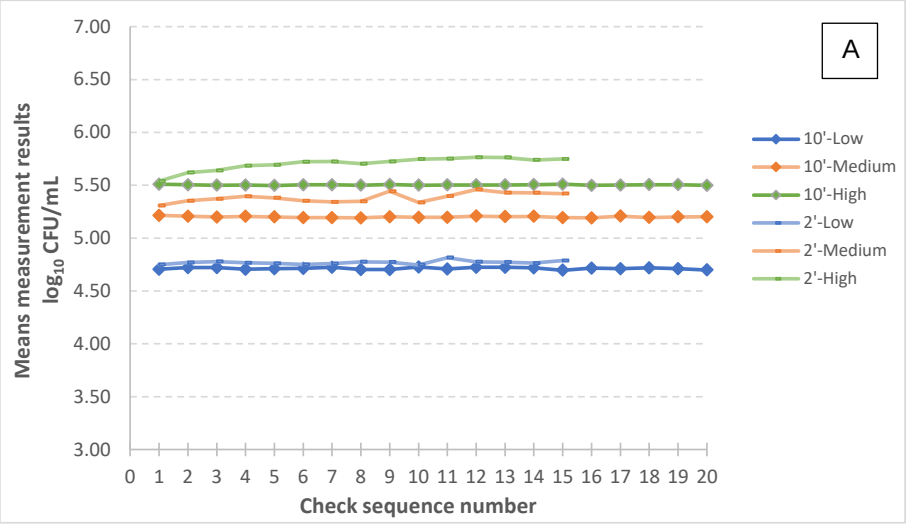
1. EVALUATION OF THE STABILITY, CARRY-OVER EFFECT BETWEEN SAMPLES, LINEARITY AND LIMIT OF QUANTIFICATION

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 2: TBC and SCC levels of the samples used for the stability evaluation (10' test / 2' test)

Level	TBC (Log_{10} CFU/ml)	SCC ($x10^3$/ml)
Low	4.7 / 4.8	79 / 77
Medium 1	5.2 / 5.4	536 / 501
Medium 2	-	1 017 / 1014
High	5.5 / 5.7	1 545 / 1 532



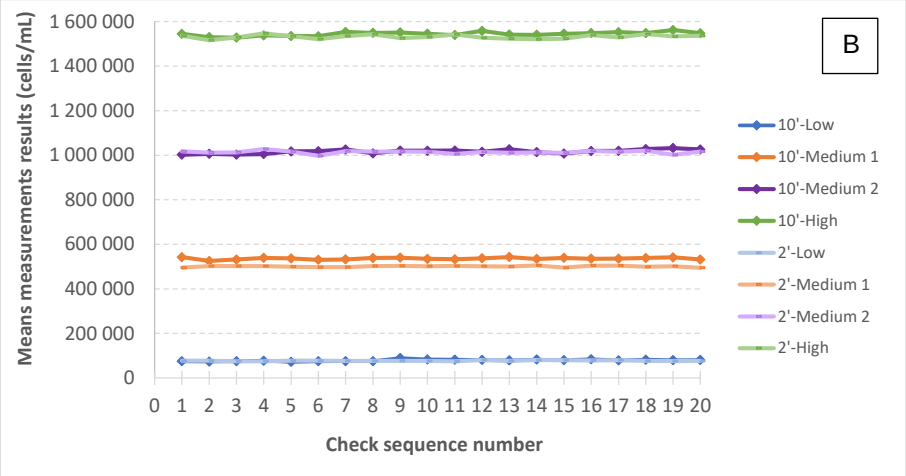


Figure 1: BactoCount™ IBCm3.0 stability for TBC (A) and SCC (B) throughout the working day

For each level, the standard deviation of repeatability (*Sr*) and the standard deviation of daily reproducibility ($S_{R,\ daily}$) were determined for the 2 programs (**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™ IBCm3.0 stability results for TBC and SCC

		10' test		2' test	
		S_r	$S_{R,daily}$	S_r	$S_{R,daily}$
TBC (Log_{10} CFU/ml)	**Low level**	0.02	0.02	0.03	0.03
	Medium level	0.01	0.01	0.02	0.05
	High level	0.01	0.01	0.02	0.06
SCC (x 10^3 cells/ml)	**Low level**	3.1	4.9	3.6	3.6
	Medium 1 level	8.7	8.7	6.4	6.4
	Medium 2 level	9.8	12.0	9.8	10.4
	High level	13.2	14.0	13.0	13.7

The results obtained during the evaluation of the stability suggest that the BactoCount™ IBCm3.0 is stable in the working day for TBC and SCC in raw cow milk and with the two programs used.

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 2000 x10^3 CFU/mL) and 3 levels for SCC (near to 500 / 1000 and 1500 x10^3 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 on blank milk samples determined for TBC and SCC

	TBC		SCC	
	10'	2'	10'	2'
Level 1	-0.19 %	-0.70 %	0.42 %	0.56 %
Level 2	0.60 %	-0.09 %	0.51 %	0.55 %
Level 3	0.60 %	0.04 %	0.51 %	0.51 %
Level 4	-0.15 %	-0.27 %	-	-
Total	**0.21 %**	**-0.25 %**	**0.50 %**	**0.53 %**

Results of carry-over between samples evaluation fulfill the requirements of 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™ IBCm3.0 was evaluated in the range from $5x10^3$ to $5x10^6$ 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 **3.8%** and **4.1%** respectively for the 10' test and the 2' test for the TBC and a ratio (r_C) determined at **0.73%** and **0.94%** respectively for the 10' test and the 2' test for the SCC, the instrument fulfills to the limits defined in the ISO 16297 for TBC (**< 5%**) and ISO 8196-3 for SCC (**< 2%**).

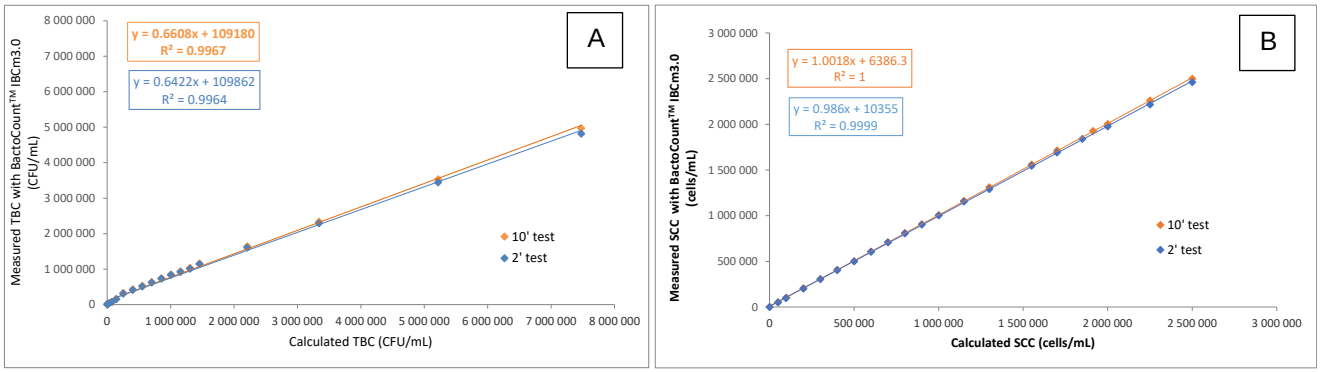


Figure 2: Linearity of the BactoCount™ IBCm3.0 for measurement of TBC (A) and SCC (B)

For TBC, the lower limit of quantification was defined at 4 600 CFU/mL (10' test) and 3 700 CFU/mL (2' test). The measurements are linear up to $5x10^6$ CFU/mL.

For SCC, the lower limit of quantification was defined at 6 100 cells/mL (10' test) and 7 500 cells/mL (2' test). The measurements are linear up to $2.5x10^6$ cells/mL.

2. EVALUATION OF THE REPEATABILITY

The repeatability of the instrument was evaluated by analysing:

- For TBC: **341** and **324 raw herd bulk cow milk samples** representative for different total bacterial count levels (respectively for the 10' test and the 2' test);
- For SCC: **111** and **110 individual raw cow milk samples** and **69** and **69 raw herd bulk cow milk samples** representative for different somatic cell count levels (respectively for the 10' test and the 2' test).

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™ IBCm3.0. For interpretation of results, the samples were sorted in different ranges of concentrations in bacteria or 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 level (**Table 4**).

Table 4: BactoCount™ IBCm3.0 - Repeatability results and criteria for TBC and SCC

		Number of samples	Mean level samples	S_r / *r*	Acceptability values (ISO 16297 or ISO 13366-2)
10' test					
TBC (Log_{10} CFU/ml)	**<4.3**	78	4.0	**0.08**	0.12
	≥4.3	263	5.0	**0.05**	0.09
SCC (x 10^3 cells/ml)	**0-150**	119	61	**6**	25
	150-300	37	204	**10**	42
	300-450	12	361	**12**	50
	450-750	9	518	**13**	63
	750-1500	3	1079	**14**	126
2' test					
TBC (Log_{10} CFU/ml)	**<4.3**	103	4.0	**0.05**	0.12
	≥4.3	221	4.9	**0.04**	0.09
SCC (x 10^3 cells/ml)	**0-150**	116	67	**7**	25
	150-300	41	211	**10**	42
	300-450	8	370	**13**	50
	450-750	8	578	**14**	63
	750-1500	6	1005	**20**	126

Repeatability of the BactoCount™ IBCm3.0 for the TBC and the 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: **334** and **315 unpreserved raw herd bulk cow milk samples** (respectively for the 10' and the 2' test);
- For SCC: **111** and **108 individual raw cow milk samples** preserved with bronopol and **68** and **69 unpreserved raw herd bulk cow milk samples** (respectively for the 10' and the 2' test).

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.

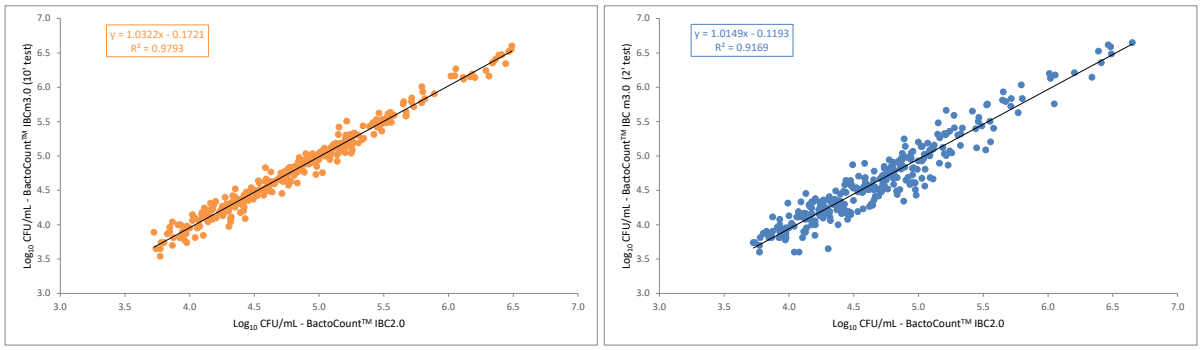


Figure 3: Relation between the BactoCount™ IBCm3.0 and the BactoCount™ IBC2.0 for TBC (● 10' test ● 2' test)

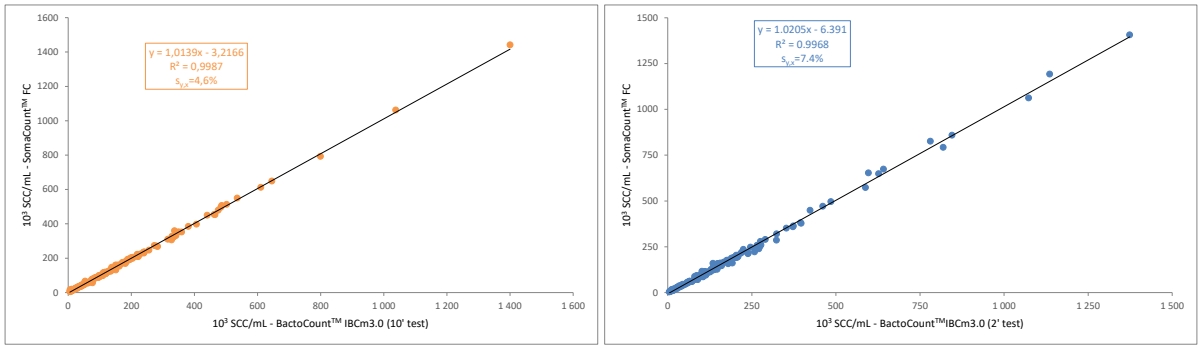


Figure 4: Relation between the BactoCount™ IBCm3.0 and the SomaCount™ FC for SCC (● 10' test ● 2' test)

For TBC, the residual standard deviation determined was **$s_{y,x}$ = 0.09** $\log_{10}$ CFU/ml for the 10' test and **0.17** $\log_{10}$ CFU/mL for the 2' test and complies with the limit of **0.40** $\log_{10}$ CFU/mL defined in the ISO 16297 and EU-RL MMP document.

For SCC, the residual standard deviation determined for the total of samples (**$s_{y,x}$ = 4.6%** for the 10' test and **7.4%** for the 2' test) is lower than the limit defined in the ISO 8196-3 (**8 %**).

The accuracy of the BactoCount™ IBCm3.0 for TBC and SCC fulfills 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™ IBCm3.0 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 SCC and TBC 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 2' test and the 10' test.

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 two programs (10' test and 2' test) of the BactoCount™ IBCm3.0 complies the standard requirements for Total Bacterial Count and for Somatic Cell Count in raw cow milk.

On the basis of this validation 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™ IBCm 3.0 – Delphine LAROSE
MicroVal Validation number: 2021LR98

STANDARDS, DRAFT STANDARDS

ISO published standards

MILK AND MILK PRODUCTS	
ISO 22662 March 2024	Milk and milk products — Determination of lactose content by high-performance liquid chromatography (reference method) *Replace ISO 22662 (September 2007)*
MATERIAUX DE REFERENCE	
ISO 33401 February 2024	Reference materials - Contents of certificates, labels and accompanying documentation *Replace ISO GUIDE 31 (February 2016)*
ISO 33407 February 2024	Guidance for the production of pure organic substance certified reference materials
METROLOGIE	
ISO 8655-10 February 2024	Piston-operated volumetric apparatus- Part 10: User guidance, and requirements for competence, training, and POVA suitability
ISO/IEC GUIDE 98-1 February 2024	Guide to the expression of uncertainty in measurement - Part 1: Introduction *Replace ISO/IEC GUIDE 98-1 (September 2009)*
QUALITY MANAGEMENT	
ISO 9001/Amd1 February 2024	Quality management systems – Requirements - Amendment 1: Climate action changes
FOOD SAFETY	
ISO 22000/Amd1 February 2024	Food safety management systems — Requirements for any organization in the food chain — Amendment 1: Climate action changes

ISO standards under development

MICROBIOLOGY OF THE FOOD CHAIN	
ISO/DIS 13136-1 May 2024	Microbiology of the food chain — Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) — Part 1: Horizontal method for the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC)
ISO/DIS 13136-2 May 2024	Microbiology of the food chain - Detection, isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) - Part 2: Horizontal method for the characterization of Shiga toxin-producing *Escherichia coli* (STEC) isolates

NEW EU REGULATIONS

CONTAMINANTS

O.J.E.U. Serie L, 5th April 2024 – Commission Regulation (EU) 2024/1003 of 4 April 2024 amending Regulation (EU) 2023/915 as regards maximum levels for the sum of 3-monochlorpropanediol (3-MCPD) and 3-MCPD fatty acid esters in infant formulae, follow-on formulae and food for special medical purposes intended for infants and young children and young child formulae
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401003

NOVEL FOOD

O.J.E.U. Serie L, 9th April 2024 – Commission Implementing Regulation (EU) 2024/1027 of 8 April 2024 amending Implementing Regulation (EU) 2017/2470 as regards the specifications of the novel food galcto-oligosaccharide
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401027

O.J.E.U. Serie L, 10th April 2024 – Commission Implementing Regulation (EU) 2024/1037 of 9 April 2024 authorising the placing on the market of monosodium salt of L-5-methyltetrahydrofolic acid as a novel food and amending Implementing Regulation (EU) 2017/2470
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401037

STANDARDS - REGULATIONS

O.J.E.U. Serie L, 10th April 2024 – Commission Implementing Regulation (EU) 2024/1046 of 9 April 2024 authorising the placing on the market of β-glucan from *Euglena gracilis* microalgae as a novel food and amending Implementing Regulation (EU) 2017/2470
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401046

O.J.E.U. Serie, 10th April 2024 – Commission Implementing Regulation (EU) 2024/1047 of 9 April 2024 authorising the placing on the market of 3'-sialyllactose sodium salt produced using a derivative strain of *Escherichia coli* W (ATCC 9637) as a novel food and amending Implementing Regulation (EU) 2017/2470
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401047

P.D.O.

O.J.E.U. Serie C, 26th January 2024 - Publication of an approved standard amendment to a product specification of a protected designation of origin or protected geographical indication in the agricultural products and foodstuffs sector, as referred to in Article 6b(2) and (3) of Commission Delegated Regulation (EU) No 664/2014 [Pecorino del Monte Poro (cheese) (PDO)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C_202401059

O.J.E.U. Serie L, 5th February 2024 – Commission Implementing Regulation (EU) 2024/443 of 29 January 2024 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Queijo de Azeitão (cheese) (PDO)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400443

O.J.E.U. Serie C, 7th February 2024 - Publication of an approved standard amendment to a product specification of a protected designation of origin or protected geographical indication in the agricultural products and foodstuffs sector, as referred to in Article 6b(2) and (3) of Commission Delegated Regulation (EU) No 664/2014 [Queso de La Serena (cheese) (PDO)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C_202401224

O.J.E.U. Serie C, 5th March 2024 - Publication of an approved standard amendment to a product specification of a protected designation of origin or protected geographical indication in the agricultural products and foodstuffs sector, as referred to in Article 6b(2) and (3) of Commission Delegated Regulation (EU) No 664/2014 [Burrata di Andria (cheese) (PGI)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C_202401948

O.J.E.U. Serie C, 6th March 2024 - Publication of an application for registration of a name pursuant to Article 50(2)(a) of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [Caciottone di Norcia (cheese) (PGI)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C_202401985

O.J.E.U. Serie C , 12th April 2024 – Publication of an approved standard amendment to a product specification of a protected designation of origin or protected geographical indication in the agricultural products and foodstuffs sector, as referred to in Article 6b(2) and (3) of Commission Delegated Regulation (EU) No 664/2014 [Caciocavallo Silano (fromage) (AOP)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C_202402490

O.J.E.U. Serie L, 30th April 2024 – Commission Implementing Regulation (EU) 2024/1227 of 23 April 2024 approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Gamoneu / Gemonedo (cheese) (PDO)]
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401227

PESTICIDES

O.J.E.U. Serie L, 22nd January 2024 – Commission Regulation (EU) 2024/331 of 19 January 2024 amending Annexes II and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for oxamyl in or on certain products
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400331

O.J.E.U. Serie L, 23rd January 2024 – Commission Regulation (EU) 2024/345 of 22 January 2024 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for desmedipham, etridiazole, flurtamone, profoxydim, difenacoum and potassium permanganate in or on certain products
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400345

O.J.E.U. Serie L, 23rd January 2024 – Commission Regulation (EU) 2024/352 of 22 January 2024 amending Annexes II and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for (Z)-13-hexadecen-11-yn-1-yl acetate, (Z,Z,Z,Z)-7,13,16,19-docosatetraen-1-yl isobutyrate, acrinathrin, azimsulfuron, famoxadone, prochloraz and sodium hypochlorite in or on certain products
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400352

STANDARDS - REGULATIONS

O.J.E.U. Serie L, 25th January 2024 – Commission Regulation (EU) 2024/376 of 24 January 2024 amending Annex II to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for indoxacarb in or on certain products

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400376

O.J.E.U. Serie L, 2th April 2024 – Commission Implementing Regulation (EU) 2024/989 of 2 April 2024 concerning a coordinated multiannual control programme of the Union for 2025, 2026 and 2027 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin and repealing Implementing Regulation (EU) 2023/731

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400989

O.J.E.U. Serie L, 16th April 2024 – Commission Regulation (EU) 2024/1077 of 15 April 2024 amending Annex II to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4-DB,iodosulfuron-methyl, mesotrione and pyraflufen-ethyl in or on certain products

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202401077

AFNOR VALIDATIONS

During the February and April meetings, the Technical Committee of NF VALIDATION approved by vote:

Commercial name	Date	Certificate	Description
RENEWALS OF VALIDATIONS			
SIMPLE METHOD FOR *SALMONELLA* (SMS)	Validation date: 7 May 2004 Renewal: 4 Apr 2024 **End of validity: 7 May 2028**	**AES-10/04-05/04**	**Detection of motile *Salmonella*** All human and animal food products, and production environmental samples (except primary production environment)
IQ-CHECK *SALMONELLA* II	Validation date: 1 Jul 2004 Renewal: 4 Apr 2024 **End of validity: 1 Jul 2028**	**BRD-07/06-07/04**	**Detection of *Salmonella* spp.** All human and animal food products and production environmental samples (including animal faeces and environmental samples from the primary production stage)
VIDAS UP *LISTERIA* (LPT)	Validation date: 10 May 2016 Renewal: 4 Apr 2024 **End of validity: 10 May 2028**	**BIO-12/33-05/12**	**Detection of *Listeria* spp.** All human food products and industrial production environmental samples
NEOGEN MOLECULAR DETECTION ASSAY 2 - *LISTERIA*	Validation date: 18. May 2016 Renewal: 4 Apr 2024 **End of validity: 18 May 2028**	**3M-01/14-05/16**	**Detection of *Listeria* spp.** All human food products and industrial production environmental samples
VIDAS *LISTERIA MONOCYTOGENES* 2 (LMO2)	Validation date: 12 Mar 2004 Renewal: 8 Feb 2024 **End of validity: 12 Mar 2028**	**BIO-12/11-03/04**	**Detection of *Listeria monocytogenes*** All human food products and production environmental samples
RAPID' *SAKAZAKII*	Validation date: 12 May 2012 Renewal: 8 Feb 2024 **End of validity: 10 May 2028**	**BRD-07/22-05/12**	**Detection of *Cronobacter* spp.** Powdered infant formula and cereals with or without probiotics
EXTENSIONS OF VALIDATIONS			
BACGENE *SALMONELLA* SPP.	Validation date: 26 Mar 2015 Extension: 8 Feb 2024 **End of validity: 26 Mar 2027**	**EGS-38/01-03/15**	**Detection of *Salmonella* spp.** All human and animal food products and production environmental samples (except primary production environment)
GENE-UP *SALMONELLA*	Validation date: 30 Jun 2016 Extension: 8 Feb 2024 and 4 Apr 2024 **End of validity: 30 Jun 2024**	**BIO-12/38-06/16**	**Detection of *Salmonella* spp.** All human food products, pet food products and industrial production environmental samples
SALMONELLA PRECIS	Validation date: 04.12.2007 Extension: 08.02.2024 **End of validity: 04.12.2027**	**UNI-03/06-12/07**	**Detection of *Salmonella* spp.** All human and animal food products and production environmental samples (except primary production environment)
THERMO SCIENTIFIC SURETECT *SALMONELLA* SPECIES PCR ASSAY	Validation date: 4 Nov 2013 Extension: 8 Feb 2024 and 4 Apr 2024 **End of validity: 4 Nov 2025**	**UNI-03/07-11/13**	**Detection of *Salmonella* spp.** All human food products, pet food, industrial production environmental samples, and primary production environment
BACGENE *LISTERIA* SPP.	Validation date: 26 Jan 2017 Extension: 8 Feb 2024 **End of validity: 26 Jan 2025**	**EGS-38/02-01/17**	**Detection of *Listeria* spp.** All human food products and industrial production environmental samples
BACGENE *LISTERIA MONOCYTOGENES*	Validation date: 26 Jan 2017 Extension: 8 Feb 2024 **End of validity: 26 Jan 2025**	**EGS-38/03-01/17**	**Detection of *Listeria monocytogenes*** All human food products and industrial production environmental samples

AFNOR VALIDATIONS

BACGENE *LISTERIA MULTIPLEX*	Validation date: 14 Mar 2017 Extension: 8 Feb 2024 **End of validity: 14 Mar 2025**	**EGS-38/05-03/17**	**Detection of *Listeria* spp. and *Listeria monocytogenes*** All human food products and industrial production environmental samples
THERMO SCIENTIFIC SURETECT *CRONOBACTER* SPECIES PCR ASSAY	Validation date: 3 Dec 2015 Extension: 8 Feb 2024 **End of validity: 3 Dec 2027**	**UNI-03/11-12/15**	**Detection of *Cronobacter* spp.** Infant formula and industrial production environmental samples

The validation certificates and the recapitulative list are available at the following website address:
https://nf-validation.afnor.org/en/food-industry/#discover-certified-methods

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Publication le 30 avril 2024 - Publication trimestrielle
Impression : ACTALIA Cecalait, B.P. 70129, 39801 POLIGNY CEDEX
Tél. : 33.(0)3.84.73.63.20 - Fax : 33.(0)3.84.73.63.29
Dépôt légal : à parution
ISSN : 1298-6976
Prix : 11.87 € HT