

DEVELOPMENT OF A SECONDARY CRM FOR SOMATIC CELL COUNTING FOR USE IN MILK QUALITY CONTROL LABORATORIES

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INTRODUCTION

The dairy industry relies on reliable data to make timely decisions regarding the quality and safety of raw materials. Therefore, laboratories must be technically competent and ensure the validity of their results through metrological traceability. Traceability to a common and stable international reference is essential for the comparability and global acceptance of results. To ensure this, the use of Certified Reference Materials (CRM) is crucial.

This requires the development of regional capabilities to produce CRM in the dairy sector, contributing to obtaining valid results and improving competitiveness in global markets.

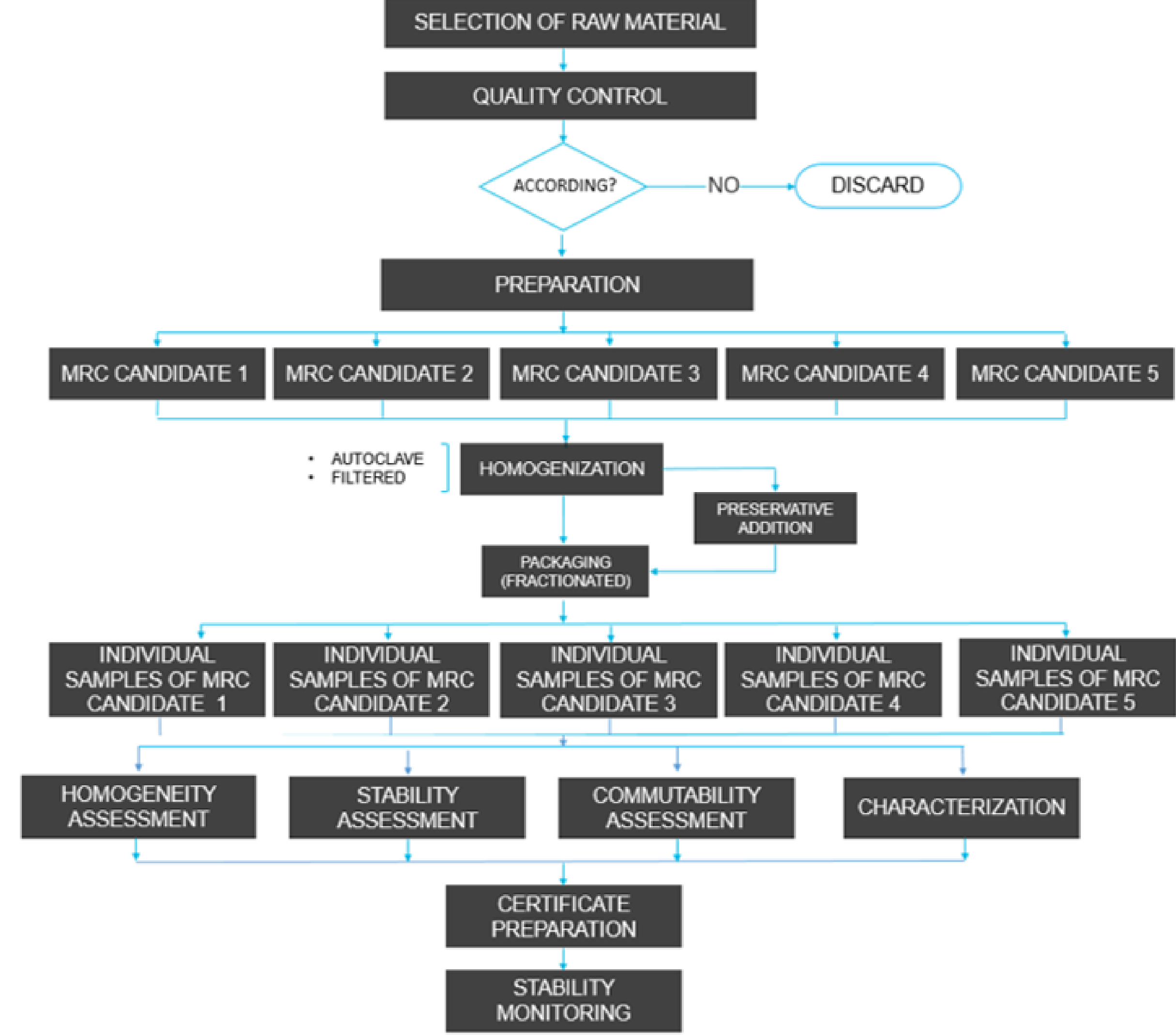
In Argentina, the Laboratorio de Materiales de Referencia of INTI-Lácteos designs, produces, and distributes CRM in various dairy matrices. These CRM are utilized by national and Latin American laboratories for the calibration of measurement instruments and the evaluation of analytical methods, ensuring the validity of results. An example of this is the CRM for somatic cell counting (SCC) in milk, which is used to assess milk quality and safety.

OBJECTIVE

To produce a secondary CRM for SCC, INTI-MRC002, in a matrix similar to natural milk, traceable to the International System of Units (SI) through the certified values of the primary CRM ERM®-BD001, which has been marketed since 2020 by the Joint Research Centre.

METHODOLOGY

Figure 1.
Schematic diagram of the INTI-MRC002 production process.

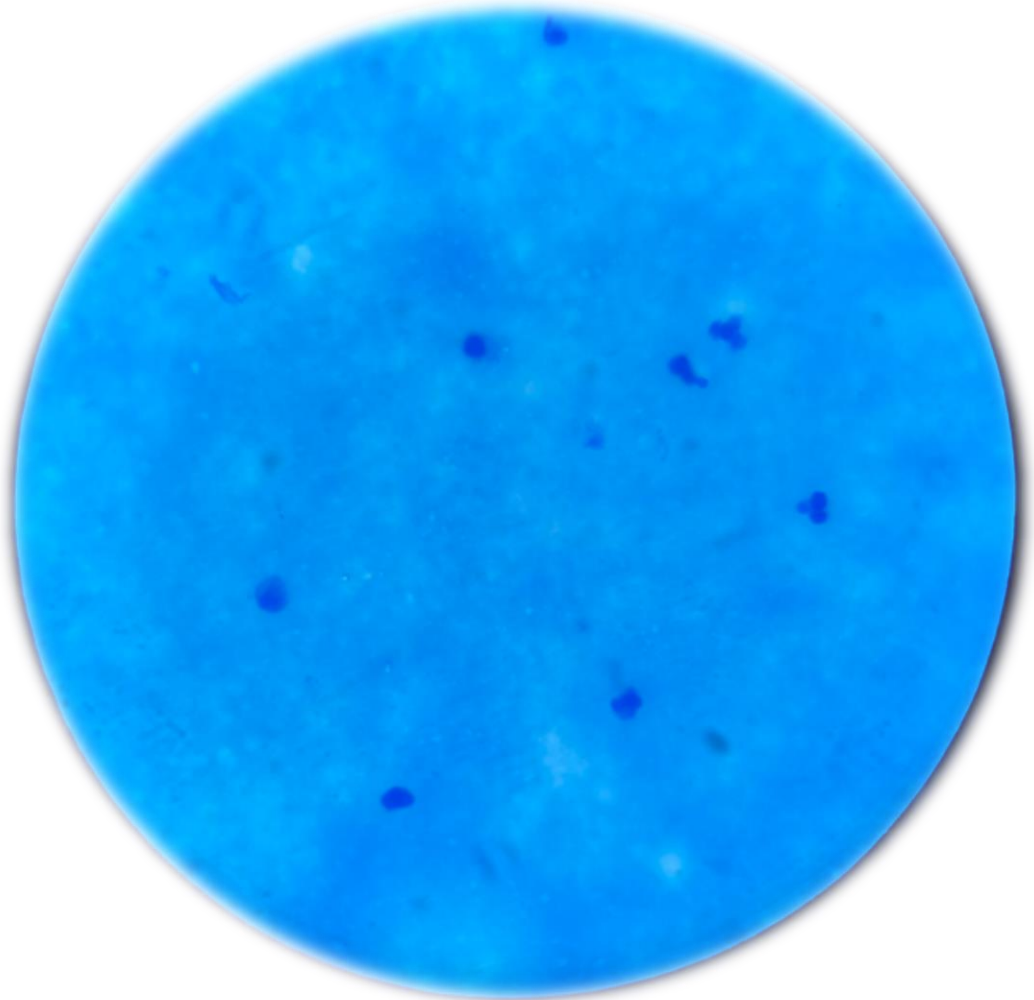


The analytical methods utilized for assigning values adhere to the ISO-IDF Standards.

ISO 13366-1 | IDF 148-1:2008. Milk – Enumeration of somatic cells – Part 1: Microscopic method (Reference method). The Laboratory is accredited by the Organismo Argentino de Acreditación (OAA) for its application of this method.

ISO 13366-2 | IDF 148-2:2006. Milk – Enumeration of somatic cells – Part 2: Guidance on the operation of fluoro-opto-electronic counters. These measurements are conducted in external subcontracted laboratories, accredited by the OAA.

The developed CRM included five levels of SCC, covering concentrations from 50000 cells/ml to 1200000 cells/ml. Its homogeneity was studied, and its long-term stability was evaluated for 62 days. The trueness of the reference measurement procedure was demonstrated by analyzing ERM®-BD001. The characterization involved the use of the reference method in the producing laboratory and the fluoro-opto-electronic method in competent laboratories, assigning property values and estimating their associated uncertainties.



RESULTS

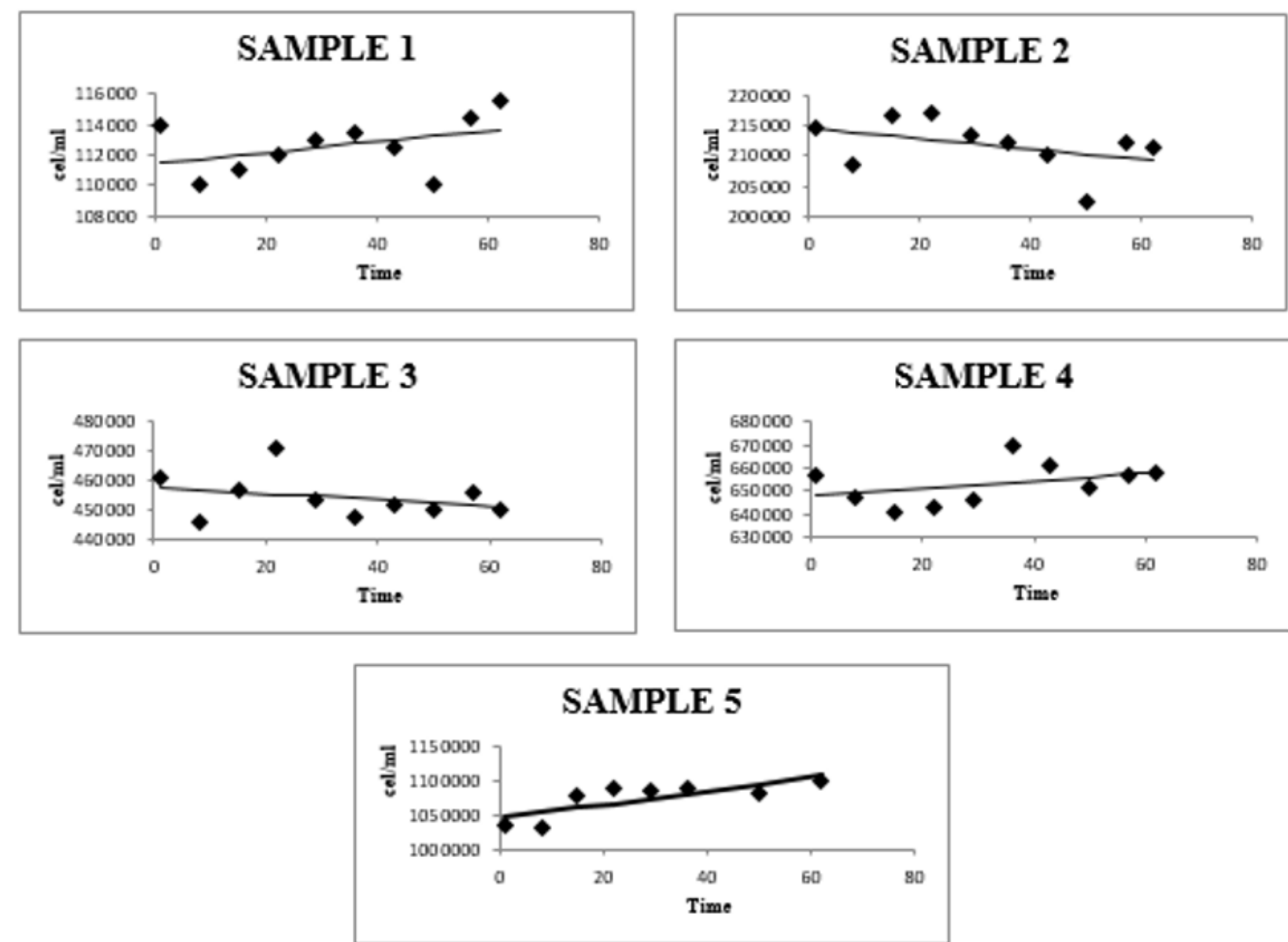
Homogeneity Study

The homogeneity study involved the Grubbs test to identify outliers. A linear regression analysis confirmed the absence of trends in packaging and measurement sequences, with acceptance criteria requiring the confidence interval of the slope to include zero. Homogeneity was further assessed using one-way ANOVA, as shown in Table 1. In all cases, the calculated F-value was lower than the critical F-value, confirming that the mean SCC values across the five analyzed units were equal. These results validate the homogeneity of the SCC property in the material.

Stability Study

Figure 2.

Long-term stability study.



A long-term stability study was conducted, performing 2 measurements at each time point (1, 8, 15, 22, 29, 36, 43, 50, 57, and 62 days), under storage conditions of 4 °C ± 2 °C.

Table 2 presents the results of the analysis of variance, while Figure 1 illustrates the absence of trends during the evaluated period and conditions. As a result, no significant degradation was observed, demonstrating the material's stability under these conditions.

Table 1.
Results of the trend analysis and homogeneity for the 5 sublots.

	ANALYZED TREND				TEST ANOVA		UNCERTAINTY
	Measurement trends (analyzed)		Processing trends (packaging)		F _{Calculated}	F _{Critical}	u _{hom} (cells/ml)
	slope interval		slope interval				
	Lower	Upper	Lower	Upper			
	1	-668,6	479,8	-712,5	425,8	0,33	4,387
2	-1762	1531,3	-1574,4	1721,3	0,124	4,387	5 843
3	-2266,4	1476,1	-1139,7	2517,3	0,142	4,387	6 659
4	-2306,6	2474,4	-3092,4	1567,9	1,397	4,387	5 018
5	-4765	2030,8	-4206	2786,4	1,017	4,387	1 673
	No significant trends were		No significant trends were		F _{Calculated} < F _{Critical}		

Table 2.
Data obtained from the analysis of variance for the study of stability.

SAMPLE	REGRESSION DATA		u _{Its} (cells/ml)
	F _{calculated}	F _(1,n-2, α)	
1	1,546	5,32	1 796
2	1,703	5,32	4 022
3	0,85	5,32	7 376
4	1,597	5,32	8 472
5	2,289	6,61	45 925



Trueness Assessment

Analysis of the ERM®-BD001 CRM demonstrated that the measurements and property values are mutually consistent within their respective uncertainties.

Material Value Assignment and CRM Uncertainty Estimation

Table 3.
Certified values and uncertainties.

INTI-MRC002-G2304	SCC (cells/ml)	Expanded Uncertainty U (cells/ml)
SAMPLE N°		
1	104 000	30 698
2	356 000	65 762
3	494 000	74 560
4	711 000	86 634
5	905 000	134 502

Each assigned value was calculated by weighed the averages of the results obtained according to both the reference method (x_{LMR}) and the routine method (x_{LS}).

The uncertainty of the assigned value was estimated by a quadratic combination of the standard uncertainty from the characterization (u_{char}), the standard uncertainty provided by the homogeneity study (u_{hom}) and the standard uncertainty provided by the long-term stability study (u_{Its}). A coverage factor k = 2 (95 % confidence) was applied to obtain the expanded uncertainties.

CONCLUSIONS

- Has achieved the development of a secondary CRM in SCC, ensuring metrological traceability of the values assigned to the SI through the certified values of the primary CRM ERM®-BD001.
- This development has enabled reaching a higher common link in the metrological traceability chain, allowing comparability of results and achieving universal acceptance. This is how INTI user laboratories employing our secondary CRM for the calibration of their fluoro-opto-electronic equipment will obtain measurement results traceable to the SI, which can be compared with results from other laboratories, enhancing acceptance and mutual trust in the dairy sector.
- The expectation is to promote the development of secondary CRMs to achieve better equivalence of results for properties of interest in the dairy industry. This will require international collaboration in the development of primary CRMs with assigned values in operationally defined measurands.

REFERENCES

- EC Joint Research Centre. (2020) Certification report. The certification of the concentration of somatic cells (somatic cell count, SCC) in cow's milk: ERM®-BD001. Available through: <https://crm.jrc.ec.europa.eu/p/ERM-BD001> (Visited January 2024).
- International Organization for Standardization ISO (2016). General requirements for the competence of reference material producers (ISO Standard No.17034:2016).
- ISO (2017). Reference materials – Guidance for characterization and assessment of homogeneity and stability (ISO Guide 35:2017).