ARTICLE

EVALUATION OF THE DELTA INSTRUMENTS SOMASCOPE SOMATIC CELLS COUNTER

The Somascope LFC is an automatic instrument, which permits the enumeration of somatic cells in milk. It is manufactured by Delta Instruments (Advanced group, Netherlands) and distributed by Humeau in France.

This instrument uses the fluoro-opto-electronic method. The test portion is mixed to a dye (DAPI) in order to disperse fat globules and to dye somatic cell nuclei. An aliquot is injected into a laminar flow carrier fluid. The cells stained are separated by the flow and are exposed to the light beam of a Light Emitting Diode (LED) and emit a yellow fluorescence light directed toward 2 detectors. Only impulses, whose the intensities are beyond to a fixed threshold (in mV) and received by both detectors, are counted and converted in term of cellular concentration using a calibration equation.

This apparatus, which is connected to a computer that ensures the signal treatment, can be associated to the "Lactoscope" infrared analyser to constitute the "Combiscope".

The evaluation tests were performed in ACTALIA Cecalait's physico-chemistry laboratory (reference analyses and implementation of the Somascope) from February to June 2013. After preliminary tests of stability of the instrument, contamination between samples, linearity and calibration, the repeatability and accuracy were evaluated in cow, goat and ewe milks.

A cleaning solution (aqueous solution of Decon® at 4 %), a carrier fluid (aqueous solution of triton X100® at 0,1 %), and a dye solution (ref S000070000 kit) were necessary for these tests.

The apparatus was configured for a rate of 600 samples per hour and no correction of contamination.

The appreciation criteria of the estimated parameters were taken from ISO 8196-3 / IDF 128-3:2010, or from the CNIEL/IE handbooks concerning the use of somatic cells counters with the context of milk payment and milk control in France (CNIEL PROC CE 04).

A. PRELIMINARY TESTS

A.1. Evaluation of the stability

The stability was evaluated by the analysis, in automatic mode, of milk every 20 minutes, representing 20 measurement cycles. This evaluation was performed on 3 levels of cellular rates 3 times per level. To evaluate the stability of the instrument, the repeatability and reproducibility were calculated for each level..

The standard deviation of reproducibility values (3.1 ; 2.2 and 2.1) are in accordance with the ISO 8196-3 / IDF 128-3 standard (SR ≤ 5 %).

A.2. Evaluation of contamination between samples

This criterion was evaluated in automatic analysis mode, by analysing the same cow milk and distilled water according to the sequence: MILK - MILK – WATER – WATER repeated twenty times on 3 levels of cellular rates.

The contamination rates between successive samples are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 and the CNIEL PROC CE 04 handbook (Tc ≤ 2 %).

A.3. Evaluation of linearity

The samples were prepared by mixing the proteic retentate and filtrate obtained by tangantial ultrafiltration (cutoff threshold: 0,8µm). A range of 20 levels, regularly distributed from 0 to 2500.10^{E}03 cells/ml, was performed by weighing and the corresponding dilutions were calculated using the conversion of the densities.

Each level was analysed 5 times in manual mode. The Ar/At ratio (Ar and At: amplitude of residues and amplitude of content respectively) equal to 2.54 % is higher than the recommendations (2 %) of the ISO 8196-3 / IDF 128-3 standard. This value can be explained by a dispersion of the results rather than a linearity defect. A F test, which compare the linear and order 2 curvilinear regression, confirms this analysis (Fobs = 1.70 < F (0.95,18,63) = 1.77 at 5 % risk)

The linearity of the instrument is satisfactory for the 0-2500.10^{E}03 cells/ml range.

A.3. Evaluation of the calibration

The evaluation of the calibration, initially installed by the manufacturer, was performed with 10 commercial somatic cells reference materials (SRMs) produced by ACTALIA Cecalait in June 2013. Each sample was analysed in duplicate.

The mean bias (1.20 %) and the regression slope (1.005) are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard and the CNIEL PROC CE 04 handbook (respectively +/-5 % and 1 +/-0.05).
Moreover the residual standard deviations of linear regression obtained (13.8.10^{E}03/ml) is in accordance with the recommendations of the CE 04 (Sy,x ≤ 20.10^{E}03/ml).

B. EVALUATION OF REPEATABILITY AND ACCURACY

B.1. The samples

The tests were performed using:
- for cow milk: 80 samples of of herd milk from the Franche-Comté region and 120 samples of individual milk from 4 farms in the Jura. Bronopol was added to the individual milk samples to give a final concentration of 0.02 %.
- for goat milk: 100 samples of herd milk from the Poitou-Charentes region.
- for ewe milk: 80 samples of herd milk from the Roquefort sur Soulzon region.

B.2. Procedure

The repeatability and accuracy of the instrument were evaluated using all the samples (herd and individual milk samples). The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 20 samples according to the following sequence: Set 1 rep 1 - Set 1 rep 2 - Set 2 rep 1 - Set 2 rep 2 … Set n rep 1 - Set n rep 2. A control milk was analysed every 20 samples to verify the stability of the analyser.

The accuracy evaluation concerns the values obtained after calibration and adjustment of the instrument with commercial SRMs produced by ACTALIA Cecalait. The reference method ISO 13366-1: 2007 (for cow and ewe milk, and according to the informative annex for goat milk) was used for the enumeration of the somatic cells by microscope counting (simple measurement confirmed if important residue).

B.3. Results

B.3.1. Cow milk

The following tables and figures present the results obtained:

Somatic cells range (10^E03/ml)	n	Min (10^E03/ml)	Max (10^E03/ml)	M (10^E03/ml)	Sx (10^E03/ml)	Sr (10^E03/ml)	Sr (%)	r (10^E03/ml)
Global	77	75.5	1067.0	265.6	145.1	10.0	3.77	28.1
Inf 100	5	75.5	97.0	83.6	10.9	7.8	9.34	21.9
101-1000	71	104.0	500.5	267.1	106.3	9.8	3.66	27.4
1001-2000	1	1067.0	1067.0	1067.0				

Table 1: Somascope repeatability criteria in cow herd milk samples

Somatic cells range (10^E03/ml)	n	Min (10^E03/ml)	Max (10^E03/ml)	M (10^E03/ml)	Sx (10^E03/ml)	Sr (10^E03/ml)	Sr (%)	r (10^E03/ml)
Global	114	5.0	1859.0	174.5	292.8	12.5	7.19	35.1
Inf 100	71	5.0	99.0	43.4	26.7	3.8	8.69	10.6
101-1000	41	106.0	998.5	324.0	245.9	10.7	3.30	29.9
1001-2000	2	1672.0	1859.0	1765.5	132.2	78.2	4.43	219.1

Table 2: Somascope repeatability criteria in cow individual milk samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr %: absolute an relative standard deviation of repeatability; r: maximum deviation of repeatability on 95 % of cases

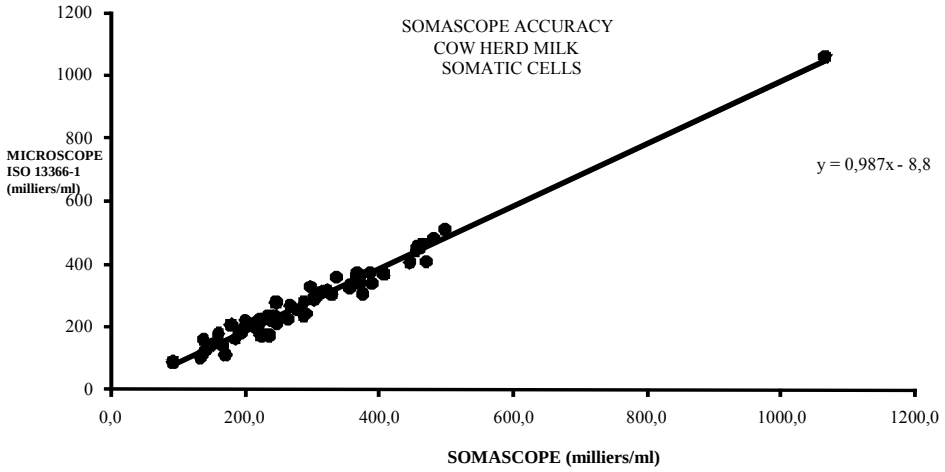
It can be noted that:

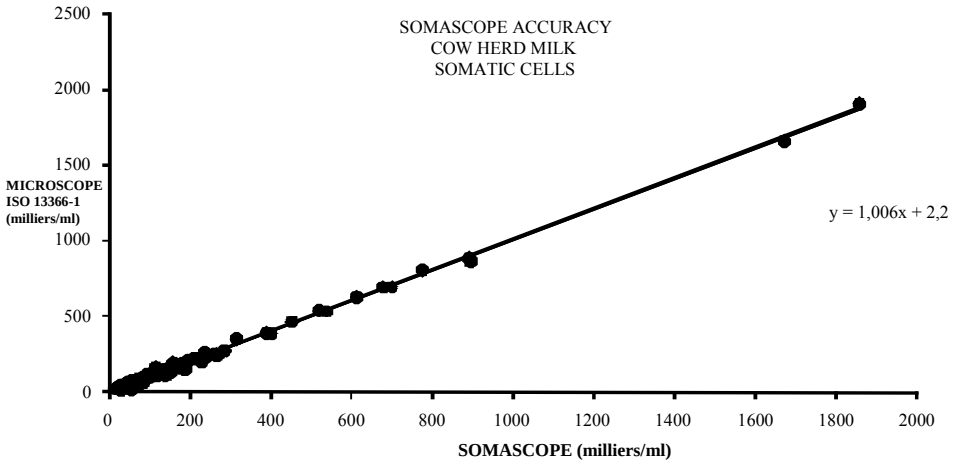
↳ For herd milk: the global relative standard deviation of repeatability (3.77 %) is in conformity with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sr ≤ 4 %) and of the CNIEL PROC CE 04 handbook (Sr ≤ 6 %). For the median range (101-1000.10^E03/ml), corresponding to the majority of the results, the relative standard deviation of repeatability obtained (3.66 %) is also in accordance with the recommendations of ISO 8196-3 / IDF 128-3 standard (Sr ≤ 4 %).

↳ For individual milk: the acceptability limits are the same than for herd milk. The global relative standard deviation of repeatablity (7.19 %) is higher than the limits due to a lot of very low results. However, for the median range (101-1000.10^E03/ml), which represents the most currently results observed, the relative standard deviation of repeatability (3.30 %) is in conformity.

	n	min (10^E03/ml)	max (10^E03/ml)	Y (10^E03/ml)	Sy (10^E03/ml)	d (10^E03/ml)	Sd (10^E03/ml)	Sy,x (10^E03/ml)	Sy,x (%)
Somatic cells Herd milk	62	91.0	1062.0	285.0	142.8	12.7	24.0	24.1	8.11
Somatic cells Individual milk	97	17.0	1907.0	195.7	301.0	-3.3	16.3	16.3	8.45

Table 3: Somascope accuracy in cow milk sample
n, min, max: number of results, minimum and maximum values; Y: mean results using the reference method; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation of the linear regression (instrument vs reference).





Figures 1 and 2: Relation between Somascope and reference results in cow milk

It can be noted that:

↳ For herd milk: the mean and standard deviation of deviations are respectively equal to 12.7 and 24.1 10^E03 cells/ml. The regression slope (0.987) and the intercept (-8.8) obtained are not significantly different from respectively 1.0 and zero (P = 5 %). The residual standard deviation of regression (24.1 $.10^E03$ cells/ml = 8,11 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sy,x ≤ 10 %).

↳ For individual milk: the mean and the standard deviation of deviations are respectively equal to –3.3 and 16.3 10^E03 cells/ml. The regression slope (1.006) and the intercept (2.2) obtained are not significantly different from respectively 1.0 and zero (P = 5 %). The residual standard deviation of regression (16.3 $.10^E03$ cells/ml = 8,45 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sy,x ≤ 10 %).

For cow milk, the results obtained are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard.

B.3.2. Goat milk

The following tables and figures present the results obtained:

Somatic cells range (10^E03/ml)	n	Min (10^E03/ml)	Max (10^E03/ml)	M (10^E03/ml)	Sx (10^E03/ml)	Sr (10^E03/ml)	Sr (%)	r (10^E03/ml)
Global	96	641.5	2492.5	1499.1	360.8	42.2	2.82	118.2
Inf 100	0							
101-1000	7	641.5	920.5	799.1	107.7	14.2	1.78	39.9
1001-2500	89	1010.0	2492.5	1549.9	317.1	40.5	2.61	113.4

Table 4: Somascope repeatability criteria in goat milk samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr %: absolute an relative standard deviation of repeatability; r: maximum deviation of repeatability on 95 % of cases

The global relative standard deviation of repeatability (2.82 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sr ≤ 4 %) and the CNIEL PROC CE 04 handbook (Sr ≤ 3 %). For the high range (1001-2500.10^E03/ml), corresponding to the majority of the results, the relative standard deviation of repeatability (2.61 %) is slightly higher than the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sr ≤ 2 %).

	n	min (10^E03/ml)	max (10^E03/ml)	Y (10^E03/ml)	Sy (10^E03/ml)	d (10^E03/ml)	Sd (10^E03/ml)	Sy,x (10^E03/ml)	Sy,x (%)
Somatic cells	60	662.0	2466.0	1402.9	362.8	22.8	52.3	52.7	3.70

Table 5: Somascope accuracy in goat milk sample
n, min, max: number of results, minimum and maximum values; Y: mean results using the reference method; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation of the linear regression (instrument vs reference).

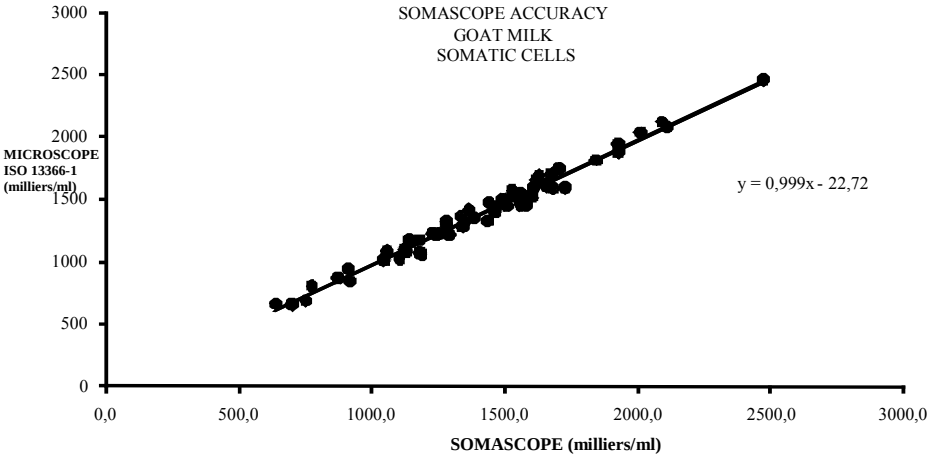


Figure 3: Relation Somascope and reference results in goat milk samples

It can be noted that the mean and the standard deviation of deviations are respectively equal to 22.8 and 52.3 $.10^{E}03$ cells/ml. The regression slope (0.999) and the intercept (-22.2) obtained are not significantly different from respectively 1.0 and zero (P = 5 %). The residual standard deviation of regression (52.7 $.10^{E}03$ cells/ml = 3,70 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sy,x ≤ 10 %).

For goat milk, the results obtained are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard.

B.3.3. Ewe milk

The following tables and figures present the results obtained:

Somatic cells range ($10^{E}03$/ml)	n	Min ($10^{E}03$/ml)	Max ($10^{E}03$/ml)	M ($10^{E}03$/ml)	Sx ($10^{E}03$/ml)	Sr ($10^{E}03$/ml)	Sr (%)	r ($10^{E}03$/ml)
Global	73	168.5	1353.5	561.9	271.3	15.2	2.71	42.6
Inf 100	0							
101-1000	67	168.5	916.0	503.0	190.1	15.2	3.02	42.5
1001-2500	6	1032.0	1353.5	1219.1	135.4	15.4	1.26	43.1

Table 6: Somascope repeatability criteria in ewe milk samples
n: number of results; min and max: minimum and maximum values; M and Sx: mean and standard deviation of the results; Sr and Sr %: absolute an relative standard deviation of repeatability; r: maximum deviation of repeatability on 95 % of cases

The global relative standard deviation of repeatability (2.71 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sr ≤ 4 %) and the CNIEL PROC CE 04 handbook (Sr ≤ 4 %). For the median rang (101-1000.$10^{E}03$/ml), corresponding to the majority of the results, the relative standard deviation of repeatability obtained (3.02 %) is also in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sr ≤ 4).

	n	min ($10^{E}03$/ml)	max ($10^{E}03$/ml)	Y ($10^{E}03$/ml)	Sy ($10^{E}03$/ml)	d ($10^{E}03$/ml)	Sd ($10^{E}03$/ml)	Sy,x ($10^{E}03$/ml)	Sy,x (%)
Somatic cells	64	153.0	1363.0	583.6	264.2	-14.1	28.8	25.9	4.55

Table 7: Somascope accuracy in ewe milk sample
n, min, max: number of results, minimum and maximum values; Y: mean results using the reference method; Sy: standard deviation of the results from the reference method; d, Sd: mean and standard deviation of deviations; Sy,x: residual standard deviation of the linear regression (instrument vs reference).

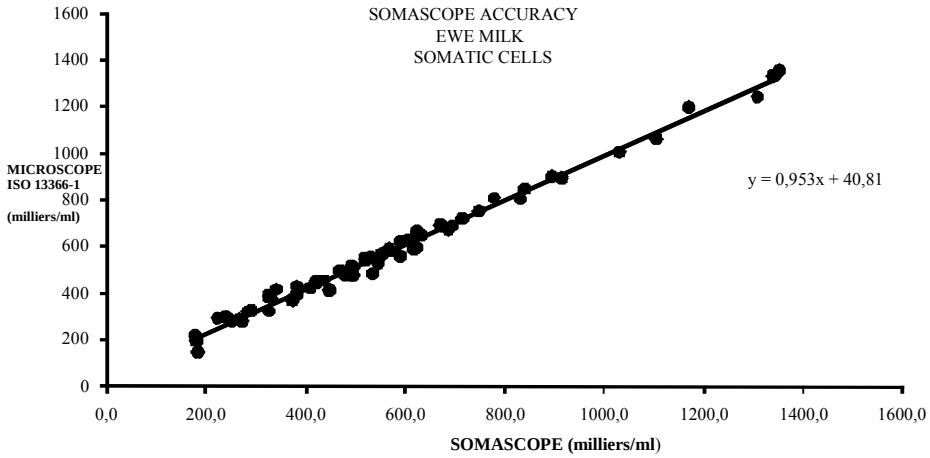


Figure 4 : Relation between Somascope and reference results in ewe milk

The mean and the standard deviation of deviations are respectively equal to –14.1 and 28.8 $10^{E}03$ cells/ml. The regression slope (0.953) is significantly different from 1.0 (P = 1 %) and the intercept (40.8) is not significantly different from zero (P = 5 %). The residual standard deviation of regression (25.9. $10^{E}03$ cells/ml = 4,55 %) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (Sy,x % ≤ 10 %).

For ewe milk, the results obtained are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard.

CONCLUSION

The results obtained during this evaluation are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3: 2010 standard and/or the CNIEL/IE handbook concerning the use of the somatic cells counter within the context of milk payment and milk control in France (CNIEL PROC CE 04) for the 3 types of milk (cow, goat and ewe).

According to the evaluation report of the Delta Intruments Somascope somatic cells counter – X. QUERVEL, P. TROSSAT – September 2013