

# EVALUATION OF SOMASCOPE® SOMATIC CELLS COUNTER

Somascope® is an instrument developed by Delta Instruments (Netherlands) for somatic cell counting in milk. Somascope® measurement principle is based on the opto-fluoro-electronic technique-combined with flow cytometry.

The physico-chemistry laboratory of CECALAIT has evaluated the analytical and instrumental characteristics of Somascope® for the first stage (Phase 1 assay in October 2001) of the process for official authorization of use for milk payment purposes .

Carry-over and linearity characteristics of the apparatus comply with the regulation requirements. Repeatability and accuracy values qualify the instrument for use by milk payment and milk recording laboratories.

## PRINCIPLE AND DESCRIPTION

Somascope® is an automated instrument dedicated to somatic cell counting in milk. It works using the opto-fluoro-electronic technic combined with flow cytometry. It is monitored by a micro-computer which deals with signal processing, instrument and calibration fittings and data recording. The instrument consists of two separate parts, the counting part and the Sample Preparation Unit.

The test portion is diluted in a reagent mixture i.e. buffered solution, detergent and fluorescent dye DAPI (4', 6-Diamidine- 2' -phenylindole dihydrochloride) in order to disperse fat globules and to dye somatic cell nuclei. By mean of a syringe, an aliquot of dyed cell suspension is injected into a laminar flow carrier fluid in a capillary tube. The cells stained with the dye and separated by the flow are exposed to the light beam of an halogen lamp and consequently emit a fluorescent light.

In order to have a clear separation between the background noise and the signal emitted by somatic cells, for each sample, the Somascope® makes a measurement at 2 different excitation wavelengths (towards 2 different photomultipliers). Only impulses arriving to both photomultipliers situated beyond a fixed discrimination level (mV) (threshold) are counted and converted in term of cellular concentration using a calibration equation.

## TESTS PERFORMED

At the rate of 360 determinations / h, the tests carried out dealt with the evaluation of stability, carry-over effect, linearity, repeatability and accuracy of the apparatus.

The evaluation criteria for the parameters estimated were taken from "148 A : 1995 IDF standard - Milk - Enumeration of somatic cells" or from the "CNIEL, Manuel des procédures de suivi des appareils de dénombrement des cellules, 2000, Réf. CNIEL Proc CE-03-05/00 = Manual for the follow through of procedures of instruments for the enumeration of cells".

## STABILITY

The evaluation of stability was performed through the analysis of three milk samples with somatic cell contents of about 500, 1000 and 1500 x 10<sup>3</sup> cell / ml respectively. Milk samples were automatically analysed, in duplicate, every 20 minutes over a half-day (i.e. 14 measurements cycles).

The results obtained showed a good stability of the instrument throughout the test period. The relative standard deviation of reproducibility (SR %) obtained are :

- 2.81 % for level 1
- 2.76 % for level 2
- 3.61 % for level 3

Those values are all smaller than 5 %, which is the maximal value accepted by IDF standard 148 A:1995 as a variation coefficient for enumerating one milk control over a day's work.

## CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing automatically one batch of milk and a skim milk safe of somatic cells (obtained by microfiltration), according to the following sequence, 20 times:

MILK - MILK - FILTRATE - FILTRATE.

The carry-over effect was assessed for three different cell levels, the correction factor for carry over being set at 0 in the instrument.

The percentage of carry-over (Tc) was estimated with the following formula :

$$Tc (\%) = [\sum (\text{filtrate } 1) - \sum (\text{filtrate } 2)] / (\sum (\text{milk } 2) - \sum (\text{filtrate } 2)) \times 100$$

## Results

Contamination between successive samples appears to be about 0,5 %, whatever the cells level of sample tested.

Those levels comply with the 1 % acceptability limit, applying to methods used for the rapid determination of milk quality (fat and protein content), but also for cell count within the same context.

## LINEARITY

Linearity was evaluated using a set of 21 evenly distributed milk with cells levels from 0 to 2100 x 10<sup>3</sup>/ml, that was automatically analysed in increasing order then decreasing order of cell levels at the rate of 3 repetitions per level. This set was elaborated by recombination with retentate and microfiltrate, using weighing and (v/v) dilution ratio calculation with respective volumetric mass.

Then linearity was assessed by plotting on a graph the residues distribution of the linear regression (ordinate) in comparison with cells rates (abscissis).

For the whole sample set, residuals were randomly distributed on the regression line with no apparent curving. Thus it can be concluded that the linearity is satisfactory.

## REPEATABILITY

Repeatability was evaluated using 131 individual cow milk samples collected from 7 dairy herds of Jura county.

Samples contained bronopol (0.02 %). Measurements were performed in consecutive duplicates, by analysing each set of 20 samples in an automated mode, 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.

Table 1 shows the results obtained.

Table 1 : Evaluation of repeatability of Somascope®

Range (10 <sup>3</sup> / ml)	n (10 <sup>3</sup> / ml)	M (10 <sup>3</sup> / ml)	Sr (10 <sup>3</sup> / ml)	Sr (%) (10 <sup>3</sup> / ml)	r (10 <sup>3</sup> / ml)
0 - 100	53	53	3.5	6.62	9.8
100 - 200	21	138	5.7	4.11	15.7
200 - 400	22	280	8.0	2.86	22.2
400 - 750	12	565	7.9	1.39	21.8
750 - 1500	16	1002	21.0	2.10	58.3
1500 - 3000	4	2213	48.8	2.21	135.2
0 - 3000	128	340	12.5	3.68	34.7

*n* : number of results

*M* : mean results

*Sr et Sr (%)* : relative and absolute standard deviation of repeatability

*r* : maximal deviation of repeatability in 95% of occurrence

## Results

Table 1 shows that the Somascope® offers a repeatability that complies with the requirements of the IDF standard 148A:1995, i.e. the estimate of the mean relative standard deviation lower than 5 %.

## ACCURACY

The accuracy was estimated using the mean  $\bar{d}$  and the standard deviation *Sd* of differences  $d = x - y$  and the residual standard deviation of regression *Sy,x* using :

- the reference method in ordinate *Y*,
- the Somascope® in abscissis *X*.

The accuracy was evaluated using 100 individual cow milk samples out of 131 collected from 7 dairy herds of Jura county. First, analyses with Somascope® were performed in duplicates with a beforehand calibration between 0 and 1.800.000 cells/ml using 9 secondary reference materials manufactured by CECALAIT. Straight after the reference method DMSCC described in IDF standard 148 A:1995 – Part 1 was applied in single test. Second DMSCC countings were performed in case residuals after regression were too large. (DMSCC : Direct Microscopy Somatic Cell Counting).

## Results

Table 2 shows the results obtained.

Table 2- Accuracy of Somascope®

Range (10 <sup>3</sup> / ml)	n (10 <sup>3</sup> / ml)	M (10 <sup>3</sup> / ml)	d (10 <sup>3</sup> / ml)	Sd (10 <sup>3</sup> / ml)	+ / - (10 <sup>3</sup> / ml)
0 - 100	24	74	- 2.0	13.4	+ / - 26.8
100 - 200	26	131	- 16.2	23.7	+ / - 47.4
200 - 400	19	275	- 7.5	24.0	+ / - 48.0
400 - 750	14	545	- 19.5	34.7	+ / - 69.4
750 - 2000	17	1039	- 9.9	37.3	+ / - 74.6
0 - 2000	100	357	- 10.5	26.7	+ / - 53.4

*n* : number of results

*M* : mean of the Somascope® values

*d* : mean of the differences between Somascope® and reference values

*Sd* : standard deviation of the differences (Somascope® - reference)

The regression line obtained between 0 and 2.000.000 somatic cells/ml shows a good adjustment of calibration.

The estimated equation is :

$$Y = 0.996 \times (\text{Somascopé}^{\text{®}}) + 12$$

with  $S_{y,x} = 26.8$  and the mean of deviation :  $d = -10.5$

The bias estimated of about 10.000 cells/ml represents 3 % in relative value, and remains within the limits authorised by the uncertainty of the reference method.

The estimation of mean accuracy of the method is +/- 53.000 cells/ml in the range of 0 to 2.000.000 cells/ml.

## **CONCLUSION**

The Somascopé<sup>®</sup> has provided satisfactory results for each aspects tested : stability, carry-over effect, repeatability and accuracy. The Somascopé<sup>®</sup> performances comply with the needs and requirements of milk payment and milk control.

Author O. LERAY

Translated by I. BECAR, O. LERAY, Ph. TROSSAT

Emended by Helen LAMPRELL

## **Bibliography**

- IDF standard 148A:1995 – Part 1 - **Milk - Enumeration of somatic cells**
- CNIEL, **Manuel des procédures de suivi des appareils de dénombrement des cellules, 2000, Réf. CNIEL Proc CE-03-05/00** = Manual of control procedures for automated somatic cell counting devices.
- QUERVEL X., TROSSAT P., **Rapport d'évaluation du Somascopé<sup>®</sup> (phase 1)**, CECALAIT, 2001, 6 p.

## **Abbreviations**

- **CNIEL** : Centre National Interprofessionnel de l'Economie Laitière = Interprofessional Centre for the Dairy Economy
- **DMSCC** : Direct Microscopy Somatic Cell Counting
- **IDF** : International Dairy Federation
- **CST** : Commission scientifique et technique = Scientific and Technical Commission

