ENUMERATION OF SOMATIC CELLS IN GOATS' MILK

The objective of this work was to study and take on board the reference method for the enumeration of somatic cells in goats' milk (FDA method), and to study the methods of transition to automatic counters.

The following work was to be carried out :

- Taking into hand the reference method in the laboratory and comparison with the cows' milk method
- Determination of the characteristics of the reference method (repeatability)
- Reflection on the calibration method of automatic counters
- Specific calibration sample analyses
- Validation of the calibration (repeatability and accuracy)

The study was carried out from April 2004 to March 2005 by CECALAIT on request of the goats' milk interprofession (ANICAP) who has ensured the financial support of this project.

1/- STUDY OF THE REFERENCE METHOD

The retained method for the cellular counts is described in the FDA document from the publication of Packard and coll. 1992. It consist in the fixing of cells, and in the coloration using methyle-pyronine Y green, followed by a microscopic counting. The reference method currently used for the enumeration of somatic cells in cows' milk, is described in the standard IDF 148. It consists in the coloration of somatic cells using methylene blue, followed by microscopic counting.

A comparative test of cellular count between the FDA and IDF 148 methods was carried out at the beginning of April 2004 on 2 bulk goats' milk samples.

It appears that the results obtained on the 2 samples using the FDA method are globally inferior, by a third, to those obtained with the IDF method.

These lower results obtained with the FDA method compared to the IDF 148 method, are similar to previous observations and could be explained by the non-selectivity of methylene blue marking the cells as cellular fragments, and by the presence of numerous non-cellular particles in goats milk which are due to the type of milky apocrine secretion (damage of the gland in the form of cytoplasmic particles). The methyl-pyronine Y green colorant does not have this inconvenience, as it fixes only particles enclosing DNA and/or RNA and allows whole cells containing intact DNA/RNA (blue-green colour) to be differentiated from cellular fragments of RNA (pink colour).

Evaluation of repeatability

The samples used are bulk goats milk from the Rhône-Alpes and Poitou-Charentes regions of France. They were collected according to the practices of the dairy interprofession for milk payment during the period from May to September 2004. Colourless bronopol at 0.02% was added and the samples were kept at 4°C until analysis (with a limit of 5 days maximum following sampling).

The analyses were carried out at CECALAIT upon reception of samples. The samples for which the values were above 3000×10^3/ml were eliminated. The results obtained are presented in the table below (10^3 cells/ml).

CLASS	0-3000	CLASS 0-1000	CLASS 1000-2000	CLASS 2000-3000
N	84	28	48	8
Sr	34	21	27	80
Sr (%)	2.8	2.8	2.0	3.3

Repeatability characteristics of the cellular enumeration (10^3/ml) in goats milk using the FDA method
N: number of results ; Sr et Sr (%): standard deviation of repeatability, absolute and relative

The results obtained present low standard deviations of repeatability of the same order of size as those obtained using the IDF 148 method with cows milk samples.

2/- STUDY OF AUTOMATED CELLULAR COUNTING

All the automated cell counting were carried out with an automatic counter, the Bentley SCC 150®,

previously calibrated using secondary reference material (SRM) cows milk samples. The technique used is flow cytometry with discrimination of the pulse-height according to a variable threshold.

The results obtained appear to be unsatisfactory, indeed the mean deviations are low (-5.10^3/ml), and the standard deviation of deviations and the residual standard deviation are high, which translates in a wide dispersion of results and an intercept significantly different from 0 (+80.10^3/ml). This dispersion is presumably linked to the accumulation of partial results over quite a long period of time (May to September 2004), as well as to the variations in calibration of the analyser over this period.

That is why, resorting to the preparation of specific goats milk SRM has been envisaged for the calibration of the analyser.

Calibration

In order to verify the usefulness of goats' milk SRMs, two series of 9 samples, of variable somatic cell content ranging from zero to 1800 10^3/ml, were realised by skimming and microfiltration of the bulk goats milk. Bronopol 0.1% was added to the samples. The series were realised in November 2004 and February 2005.

These samples were analysed in duplicate using the FDA method and by automatic counting (cows milk calibration).

Results

The results obtained are presented in the table below:

PARAMETER	SERIES 1	SERIES 2
d	3	19
Sd	31	30
b(b')	0.974 (0.997)	0.982 (0.974)
a	20	-3
Sy,x (S'y,x)	28 (30)	30 (28)

Standardisation parameters of the automatic cell counter Bentley SCC150® using goats milk SRM.
d and Sd: mean and standard deviation of deviation instrument-reference ; b et b': single linear regression gradient (Y = b.X + a) or forced by a=0 (Y = b'.X); a: intercept of the single linear regression; Sy,x et S'y,x: residual standard deviation of the single or forced linear regression by a=0.

The mean deviations are low. The regression gradients are close to 1 (non significant difference) and the intercepts are close to 0. The residual standard deviations are low.

It can be noted that the relation between the two methods is stable over the two periods tested.

Globally, the deviations being low, the calibrations proposed using goats milk samples do not appear to be statistically different from those in vigour using cows milk samples.

3/- VALIDATION OF STANDARDISATION

A comparative analysis of goats milk samples by the reference method and the automated method calibrated with cows milk samples, was carried out in order to evaluate the counting accuracy with this type of calibration.

The samples used were obtained as previously described over the period of February and March 2005.
These samples were analysed in duplicate by the FDA method and by automated counting, the instrument having previously been calibrated using cows milk SRM ranging from zero to 1800 10^3/ml.

Results

Evaluation of repeatability

The results obtained are presented in the table below (10^3 cell /ml).

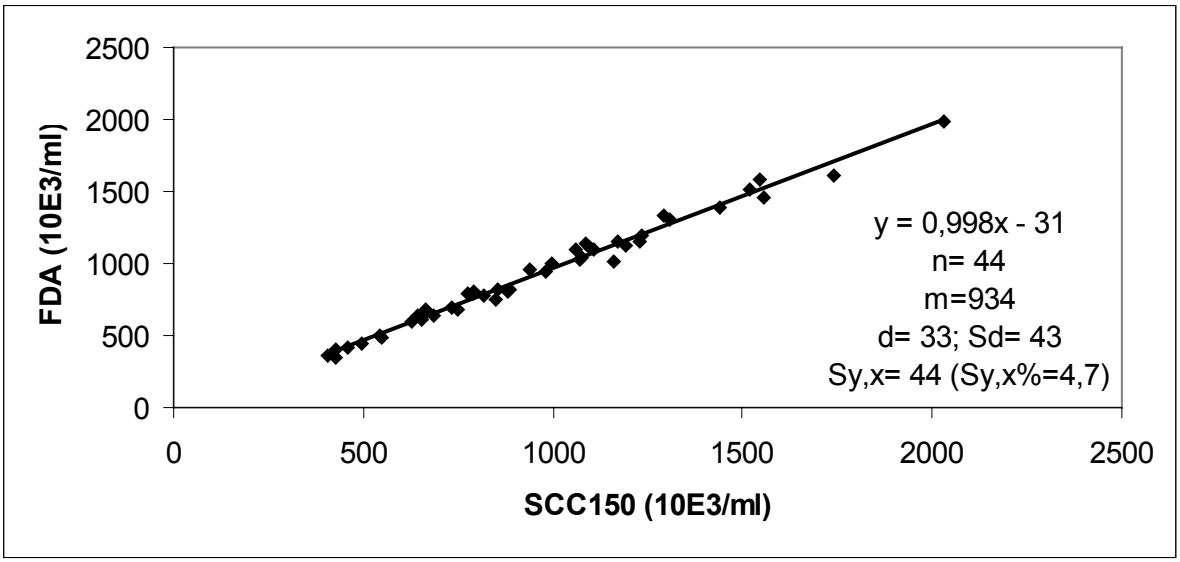
CLASS	0-2000	CLASS 0-1000	CLASS 1000 - 2000
N	44	24	20
Sr	13	9	17
Sr(%)	1.4	1.3	1.3

Repeatability characteristics of cellular enumeration (10^3/ml) of goats milk by automated counting (Bentley SCC150®)
N: number of results ; Sr et Sr(%): standard deviation of repeatability, absolute and relative.

The results obtained are good. Indeed, the standard deviations of repeatability are low and inferior to those obtained by the reference method. They are inferior to those observed when using individual cows milk samples (approximately 2.5%) and inferior to the recommended 5%.

Evaluation of accuracy

The figure below presents the results obtained:



Linear relation between the FDA method and the Bentley SCC150® on bulk goats milk samples
n : number of results, m: mean reference results ; d et Sd: mean and standard deviation of deviations instrument-reference ; Sy,x et Sy,x%: residual standard deviation of linear regression, absolute and relative

Taking into mind these results, it appears that the mean deviation of $33x10^3$/ml is superior to 0 (non-significant difference at 5%) which shows a good global concordance between results.

The gradient of 0.998 is very close to 1 (non significant difference) and the negative intercept is –31 (statistically non significant difference). This last parameter can be explained by the absence of low cell content samples (inferior to 300) which does not allow a good adjustment of the intercept.

The absolute residual standard deviation of 44 (45 for a=0) 10^3/ml can appear to be high, but when related the mean content, it is only 4.6 % (4.7% for a=0) and therefore inferior to that of 6 to 7% observed during the evaluation of the instrument with cows milk.

In conclusion, the calibration carried out using cows milk standards (wide range 1800 10^3/ml) allows a satisfactory accuracy to be assured for the enumeration of cells in goats milk.

4/- CONCLUSION AND PERSPECTIVES

Concerning the use of goats milk SRM, the results obtained do not show the use in using such specific preparations. In effect, the study showed that the calibration using cows milk standards according to the protocol in vigour, is satisfactory for the enumeration of cells in goats milk. The results can be explained for the following reasons:

- The colorant used for automatic counting (ethidium bromide) only fixes DNA in the same way as the colorant used in the reference method for goats milk (methyl-pyronine Y green). The two colorants are therefore specific to cells enclosing DNA and do not fix non cellular particles (present in goats milk). Conversely, the methylene blue used in the reference method for cows milk, colours all the particles and its usage for cows milk is possible only thanks to the quasi-absence of non cellular particles.

- The selection of pulses by the variable threshold technique allows only the strong pulses, predominantly originating from the somatic cells excluding cellular fragments, to be retained.

Thus, by extrapolation of the results obtained with the Bentley SCC150® analyser, it can be considered that the enumeration of goats milk by automated counting necessitates, a calibration of the analysers by means of cows milk samples over an approximate range of 0 to 1800 10^3/ml (including a control of linearity within this range), as well as, presumably, the use of a variable threshold mode (differentiation of pulse height). It can be thought that the results obtained are adaptable to other analysers presenting the same functionality and performances.
Finally, due to the evolution in the types of cells present in goats milk throughout lactation, it would be interesting to verify the stability of the relation between the results obtained by the goats milk

reference method and the automated counts calibrated using cows milk.

BIBLIOGRAPHY

QUERVEL X., TROSSAT Ph. **Study report on the enumeration of somatic cells in goats' milk**, CECALAIT, May2005

PACKARD VS & Coll. 1992, **"Direct microscopic methods for bacterial or somatic cells"** in Standards method for the examination of dairy products. American Public Health Association, Washington, DC, p. 309-325