Ceca Lait

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CENTRE D'EXPERTISE ET DE CONTROLE
DES ANALYSES LAITIÈRES

2002

N°41

CECALAIT
NEWSLETTER

EVALUATION OF :

EVALUATION OF THE LACTOSCOPE® INFRARED ANALYSER

Developped by Delta Instruments, the Lactoscope® is a mid-infrared Fourier transform analyser for the determination of fat, protein and lactose content in milk, as well as other criteria such as urea content equivalent (FNCV) and determination of freezing point (FP) equivalent.

The phase 1 assay took place from July to September 2001. CECALAIT evaluated the analytical and instrumental characteristics of the Lactoscope® for the determination of fat and protein content, and FP. Its basic characteristics, stability and carry-over effect, appear to be very satisfactory. Over a range of "usual" levels, it is also satisfactory for the adjustment of linearity proposed by the developper. Finally, the repeatability and accuracy values, for individual or herd milk, appear to comply with the regulations and standardization requirements.

PRINCIPLE AND DESCRIPTION

The Lactoscope® uses a mono-beam infrared system based on the Fourier transform technic and it includes an interferometer. It is coupled with a micro-computer which deals with running and signal processing, calibration and samples.

The calibration of the apparatus is carried out using a multiple linear regression calculation, from absorbances obtained at various wavelengths (usually 3 wavelengths for fat and 4 for protein, according to the IDF standard 141). This calibration is suitable for the prediction of the "usual" parameters : fat, protein and lactose, but also for the prediction of the "urea content" equivalent or for the "freezing point" equivalent.

TESTS PERFORMED

CECALAIT physico-chemistry laboratory conducted all the evaluation tests, which concerned the reference and infrared analyses for fat and protein content and FP.

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

The evaluation criteria of estimated parameters were taken from the IDF standard 141 C : 2000 - Whole milk - Determination of milkfat, protein and lactose content (guidance for the operation of mid-infrared instruments) or from the "CNIEL - Manuel d'utilisation des appareils infrarouge dans le cadre du paiement du lait en France " (= User's guide to infrared analysers for milk payment in France).

STABILITY

The evaluation of stability was performed by analysis of three milk samples covering a range of normal measurements for fat and protein content. The milk samples were analysed automatically, in duplicate, every 15 minutes over half a day (representing 14 measurement cycles) according to the actual working conditions of a milk payment laboratory.

In order to evaluate the apparatus' repeatability and reproducibility, parameters were calaculated for each analytical criterion. The more important results are shown below.

Table 1 - Summary of the reproducibility values obtained when evaluating the stability of the Lactoscope®

Levels	Fat SR (g/l)	Protein SR (g/l)	FPD R-(m°c)
1	0.08	0.06	3.87
2	0.09	0.05	3.04
3	0.10	0.06	2.95

SR : absolute standard deviation of reproducibility
R : maximum reproducibility difference in 95 % of occurences

Results

Concerning the fat and protein criteria, the mean daily values of the standard deviation of reproducilbility observed, comply with those deduced from the "Manuel d'utilisation des appareils infrarouge dans le cadre du paiement du lait en France " (SR< L / 2,58 ; L : control card limit at 99 % equals 0,7 g/l).

Concerning the FP equivalent, in the absence of standardized values, the IDF 108 B reproducibility value was used (R= 5 m°C). The reproducibility values obtained here are all lower than the IDF value.

CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing automatically one batch of milk and distilled water, 20 times in the following sequence : MILK (M1) - MILK (M2) - MILK (M3).

The measurements concerned fat and protein content, at 3 levels for each criterion, respectively (20, 20) for milk 1; (40,30) for milk 2; (60, 40) for milk 3.

NB : In order to permit the start the analysis, NaCl was added to the water sample to give a final concentration of 0,5 %.

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

$$Tc\ (\%) = [\Sigma\ (water1) - \Sigma\ (water2))\ /\ (\Sigma\ (milk1) - \Sigma\ (milk2))\ ] \times 100$$

Results

Contamination between successive samples appears to be from 0,53 % to 0,70 %, whatever the constituant and the level tested.

These levels comply with the 1 % acceptability limit, applyied to methods used for the rapid detemination of milk quality, in the context of milk payment and milk control.

LINEARITY

For fat content, the linearity was evaluated using a set of 14 evenly distributed milk samples, from 0 to 128 g/l, elaborated using a mixture of cream and skimmed milk.

For protein content, the linearity was evaluated using a set of 14 evenly distributed milk samples, from 0 to 83 g/l elaborated using a mixture of proteinic retentate and filtrate obtained by tangential ultrafiltration (cut off level : 10 KD).

The results showed that the adjustment proposed by the manufacturer is satisfactory :

- in the range corresponding to the protein criterion
- in the range corresponding to the calibration of the apparatus, (22 to 56 g/l).

However, it is possible to widen the usable area, by using a 3rd order polynomial equation to correct the results.

REPEATABILITY

For fat and protein content, the repeatability was evaluated using 131 individual milk samples from 7 breeding farms in the Jura county and 58 herd milks.

Milk samples covered a range of values which were from 17 to 85 g/l for fat content and from 25 to 43 g/l for protein content.

For the FP equivalent, the repeatability was evaluated using 58 herd milk samples.

Samples contained bronopol (0.02 %). Measurements were performed in consecutive duplicates analysing automatically each set of samples in 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.

Tables 2 and 3 show the results obtained.

Sx : standard deviation of results
Sr et Sr (%) : relative and absolute standard deviation of repeatability
r : maximal deviation of repeatability in 95% of cases
n : number of results
min and max : minimum and maximum values
M : mean results

Table 2 : Evaluation of repeatability for individual milk

INDIVIDUAL MILK					
Criterion	n	M	Sr	Sr (%)	r
Fat	131	41.37	0.08	0.20	0.23
Protein	131	33.79	0.07	0.22	0.20

Table 3 - Evaluation of repeatability for herd milk

HERD MILK						
Criterion (g/l)	n	M	Sx	Sr	Sr (%)	r
Fat	58	37.89	1.94.	0.09	0.24	0.25
Protein	58	32.95	1.26	0.07	0.20	0.19
FPD (m)C x - 1)	58	504	10.0	1.6	0.31	4.4

Results

Tables 2 and 3 show that for fat and protein matter, the analyser offers a repeatability that complies with the requirements of the IDF standard 141 C : 2000 (Sr = 0.14 g/l and r =0.4 g/l).

Concerning the FP equivalent, the repeatability value obtained is close to the repeatablity of the Thermistor cryoscope reference method (Sr = 1.4 m°C).

ACCURACY

The accuracy was evaluated using two types of sample:

- 100 individual milk samples (out of 131) from 7 breeding farms in the Jura county, to which bronopol was added (0.02 %), for the evaluation of fat and protein content,

- 58 herd milk samples from the Franche-Comté region, to which bronopol was added (0.02 %), for the evaluation of fat, protein and FP equivalent.

The reference methods used are tjose used in the context of milk payment :

- Fat - GERBER method (NF V 04 210 AFNOR standard), single analysis but with confirmation when residues are too important.

- Protein - Amido Black method (NF V 04 216 AFNOR standard), with duplicate analyses.
- FP equivalent : plateau seeking method with use of a thermistor (NF V 04 205 AFNOR standard).

Results

Tables 4 and 5 show the results obtained, respectively for fat and protein content, using individual milk and herd milk samples. Table 6 shows results corresponding to FP equivalent measurement, using herd milk samples only. (see below)

Table 4 - Accuracy of the Lactoscope® for fat

	INDIVIDUAL MILKS	HERD MILKS
n	100	58
Y (g/l)	38.82	37.71
X	38.85	37.89
Sy	8.40	1.93
d	0.03	0.18
Sd	0.46	0.34
Sy,x	0.433	0.344
b	0.981	0.980
a	0.72	0.56

Table 5 - Accuracy of the Lactoscope® for protein

	INDIVIDUAL MILKS	HERD MILKS
n	100	58
Y (g/l)	33.16	33.11
X	32.72	32.95
Sy	2.61	1.28
d	- 0.44	- 0.16
Sd	0.38	0.21
Sy,x	0.382	0.207
b	0.991	1.007
a	0.75	- 0.08

Table 6 - Accuracy of Lactoscope® for FPD equivalent

	HERD MILKS
n	55
Y (g/l)	516
X	504
Sy	10
d	- 13
Sd	3
Sy,x	2.8
b	0.927
a	49

Keys for tables 4, 5 and 6

n : number of samples
Y : mean of the results using the reference method
X : mean of the results using the Lactoscope®
Sy : standard deviation of the results using the reference method
d : mean of the differences Lactoscope® - reference method
Sd : standard deviation of the difference
Sy,x : residual standard deviation
b : slope of the linear regression equation
a : point 0 ordinate = intercept?

CONCLUSION

Stability and carry-over effect appear very satisfactory. For the range of "usual" levels, it is also satisfactory for the adjustment of linearity proposed by the manufacturer. Finally, the repeatability and accuracy values, for individual or herd milk, appear to comply with the regulations and standardization requirements.

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IDF 128 A : 1999 - Milk - Definition and evaluation of the overall accuracy of indirect methods of milk analysis, application to calibration procedure and quality control in the laboratory

IDF 141 C : 2000 - Whole milk - Determination of milkfat, protein and lactose content (guidance for the operation of mid-infrared instruments)

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Abbreviations

AFNOR = French body for standardization
CNIEL : Centre National Interprofessionnel de l'Economie Laitière = Interprofessional Centre for the Dairy Economy
IDF : International Dairy Federation
FNCV : Feed Nitrogen Conversion Value
FPD : Freezing Point Determination

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. Repeatablity 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 dihydrocloride) 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 situed 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 10^3 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 :

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

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 10^3/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 (10^3 / ml)	n (10^3 / ml)	M (10^3 / ml)	Sr (10^3 / ml)	Sr (%) (10^3 / ml)	r (10^3 / 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 then 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 Micropscopy Somatic Cell Counting).

Results

Table 2 shows the results obtained.

Table 2- Accuracy of Somascope®

Range (10^3 / ml)	n (10^3 / ml)	M (10^3 / ml)	d (10^3 / ml)	Sd (10^3 / ml)	+/- (10^3 / 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 x (Somascope®) + 12

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

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Abbreviations

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