SUMMARY OF LA LETTRE DE CECALAIT, N° 33 (2nd quarter 2000)

Some information about the procedure used in France for using a new analyser for milk payment purposes

The procedure depends on the CST, which is a special Commission of 16 members (administration, research, dairy industry…) presided by the DGAL (Direction of Food of the Ministry of Agriculture), which authorizes the use of new materials or methods for analyses made for milk payment purposes.

A manufacturer or a distributor who wishes to sell his analyser for milk payment purposes must follow the procedure described below :

- Draw up an application for an authorization by the CST and the CNIEL (interprofessional body). The file contains the directions of use, any relevant scientific and technical contribution and some information about the advantages of the new material.
- Test the analyser (phase I) by CECALAIT, at CST's request in order to evaluate its analytical characteristics.
- Validation by CST of the report of phase I tests.
- Test in routine conditions for two months, in two different interprofessional laboratories (phase II).
- Examination of the results by the CST, which then decides whether to authorize, or not, the use of the analyser for milk payment purposes.
- The decision may be issued in the French Official Journal. However, communications listing all authorized analysers are issued regularly (the latest on 1997/7/30)

For abbreviations and bibliography, please see page 1 in La Lettre de CECALAIT

Evaluation of the Milkoscan 6000

Milkoscan 6000 is an automatic FTIR (Fourier transform infrared) analyser, developed and marketed by Foss, for analysis of fat, protein and lactose in milk. It can also measure other parameters such as urea or a freezing point equivalent (FPD). CECALAIT has recently evaluated its analytical characteristics (phase I assay, see above)

APPARATUS

Run by a Windows-based micro-computer for analyses and calibration, its analytical speed is 450 samples/h. Two different ways are available to develop the calibrations for the components.

- one is based upon the partial least squares regression (PLS) method, using the absorbances at the wavelengths usually taken in the multiple linear regression (MLR) method, ie 3 wavelengths for fat and 4 for protein (cf standard IDF 141 B: 1996). This calibration is called «traditional P.L.S. calibration» and predicts the concentration of fat, protein and lactose. The results are the same as the ones which would have been given by MLR.
- the other is based upon the partial least squares regression (PLS) method, using a set of absorbances from the spectrum of calibration samples. This calibration is called « P.L.S. Spectrum calibration» and predicts urea and FPD, but also fat, protein and lactose.

TESTS PERFORMED

Tests were performed from July to October 1999 for the following components : fat, protein and FPD.

The following characteristics were evaluated, according to IDF standard 141B :1996 and to the guidebook for infrared analysers issued in France by CNIEL (the milk payment body):

- stability
- carry-over effect
- linearity
- repeatability
- accuracy

❶ STABILITY

The stability was evaluated by the duplicate automatic analysis of a set of three milks, corresponding to the usual range of fat and protein, every 15 mn for half a day.

Whatever the calibration used, the results show that the standard relative deviation of reproducibility S_R is always lower than the value inferred by the IDF standard 141B, S_R < 0.27g/kg. For the FPD, the values are all lower than R=5 m°C, given by IDF standard 108.

❷ CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing the same individual milk and distilled water, 20 times, in the following sequence : milk – milk – water* - water*.

The carry-over effect (Tc %) was estimated with following equation :

Tc % = [(S(water 1) - S(water 2))/ (S(milk 2) - S(water 2))] x 100

Tc values are in the interval of **0.39% to 0.97%.**

These values comply with the maximum limit of 1% usually allowed, for instance in routine methods of determination of milk composition, used for milk payment purposes.

NB : for conductivity reasons, water samples were spiked with 0.4 % KCl, according to the manufacturer's recommendations.

❸ LINEARITY

Linearity was evaluated for each channel by automatic analysis, in duplicate, of a set of 11 milks with :

- fat ranging from 0 to 120 g/l,
- protein ranging from 0 to 83 g/l.

The analysis followed first increasing, then decreasing fat levels. Linearity was estimated on raw data, before applying the PLS coefficients.

The results show that the manufacturer's linearity adjustement is satisfactory for the whole range of fat and protein tested. However, it should be optimized by using a 3-order polynomial, for high level milk, for instance ewe's milk at the end of lactation (fat > 100g/l).

❹ REPEATABILITY

Repeatability was evaluated by automatic analysis of 140 individual milk samples and 55 herd milks, with fat ranging from 16 to 73 g/l and protein from 26 to 48 g/l. Only individual milks were preserved with 0.02% bronopol.

Each set of 10 samples was analysed in duplicate. The stability of the analyser was checked during the tests. Both methods of calibration were used.

The results are given in table 1, page 4, in « La Lettre de CECALAIT ».

Whatever the calibration used, the repeatability values comply with IDF standard 141B specifications, ie **Sr = 0.14 g/l and r = 0.4 g/l.**

For the FPD, Sr is below the limit given for the cryoscopic reference method, Sr = 1.4 m°C.

❺ ACCURACY

Accuracy was evaluated, as in ❹, by duplicate (not consecutive) automatic analysis of :

- 112 individual milk samples, preserved with 0.02% bronopol, for milk recording purpose,
- 55 herd milks, for milk payment purpose.

Reference methods used were the official methods for milk payment, ie :

- the Gerber method for fat,
- the Amido Black method for protein,

and the cryoscopic plateau seeking freezing point determination (IDF standard 108B).

For fat and protein, the instrument was calibrated using :

- « traditional PLS » from a set of 13 recombined milk samples, following the technique described by O. LERAY (1989),
- «P.L.S. Spectrum», made by Foss, without adjustement with local milks.

« PLS spectrum » was also used for FPD.

Accuracy was estimated by using :

- the mean bias to the reference values (*moyennes des écarts*),
- the standard deviation of the differences (*écarts types des écarts*),
- the residual standard deviation (Sy,x),
- the equations of the estimated linear regressions,

Tables 2 and 3, page 5, in « La Lettre de CECALAIT » show the results on individual and herd milks.

NB : (1) the values are not reported, because the range measured was too small.
For FPD evaluation in herd milks,5 samples were spiked with water (up to 3%) to enlarge the range tested.

↳ For fat, the mean biases are :

- **- 0.25 g/l** and **+ 0.28 g/l,** in « traditional PLS calibration »,
- **+ 1.07 g/l** and **+ 0.98 g/l**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

The slopes are not significatively different from 1.00 for herd milks, but are so, whatever the calibration, for individual milks. The residual standard deviations are :

- **0.667** and **0.292,** in « traditional PLS calibration »,
- **0.654** and **0.282**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

↳ For protein, the mean biases are :

- **- 0.01 g/l** and **– 0.08 g/l,** in « traditional PLS calibration »,
- **+ 0.94 g/l** and **+0.91 g/l**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

The slopes are significatively different from 1,00 whatever the calibration, for individual milks, and for herd milks using « PLS Spectrum ». It is not significatively different from 1,00 for herd milks, using « traditional PLS ». The residual standard deviations are :

- **0.340** and **0.132,** in « traditional PLS calibration »,
- **0.346** and **0.124**, in « PLS Spectrum calibration »,

respectively for individual and herd milks.

However, these deviations remain very small and still comply with users wishes.

For FPD, the residual standard deviation is about **6 m°C,** for individual milks and about **2.8 m°C,** for herd milks. So the estimation precision for these milks is about **± 5.6 m°C**.

In conclusion, for fat and protein, the analytical characteristics of Milkoscan 6000 comply with the limits fixed in IDF standard 141, ie residual standard deviation of 1.0 g/kg for individual milks and 0.7 g/kg for herd milks.
In «traditional PLS» calibration, the bias is slightly over +/- 0.15 g/kg for fat. It may come from the delay –about a month- between the preparation of the calibration samples and the analysis of herd milks. In « PLS Spectrum » calibration, mean biases are higher. This may come from the manufacturer's calibration, which was performed without local milks. A new calibration using local milk samples, as required in IDF standard 128 is recommended for fat, protein and FPD.

For FPD, the accuracy found here complies with the values specified by FOSS, ie Sy,x < 4 m°C for herd milks.

❻ GENERAL CONCLUSION

The analytical characteristics of Milkoscan 6000 : instrumental stability, carry-over effect, linearity, repeatability, accuracy, have all been found satisfactory. They comply with the requirements of milk payment purposes.
For FPD, using Milkoscan 6000 may be an economical way to screen milks before performing a cryoscopic analysis.

For abbreviations and bibliography, please, see pages 6 & 7 in La Lettre de CECALAIT

Questionnaire « microbiology » : the answers

At the beginning of this year, you received two questionnaires, one about reference samples in microbiology, the other about a future web site. A lot of laboratories replied (118 french laboratories, 37 foreign laboratories, that is to say a response of 29%). Thank you very much.

The 2nd questionnaire is still being treated. The « microbiology » questionnaire, however was fully analysed. Here are the highlights !

Very soon, as answers arrived, it was noticed that among french laboratories, interprofessional ones had a special profile. Therefore, their answers were separated from the other ones, see table 4 in La Lettre de CECALAIT.

↳ reference samples « microorganisms at 30°C »

We are thinking of developing this kind of sample first. 73% of the laboratories who answered were interested, with 86% foreign laboratories interested !

Except interprofessional laboratories, most laboratories, ie 86% (96% of french labs, 78% of foreign labs) would use these samples in order to check a plate count method. Only 28% would do it to check an analyser.

For practical details about the samples, see table 4, page 8, in « La Lettre de CECALAIT ».

↳ Further development

After these first samples, we would think of developing samples for other microflora found in dairy products. Most laboratories would be interested then in :

- coliform enumeration : 61% ,
- *Escherichia coli* and / or *Staphylococci* enumeration, and also detection of *Salmonella* and / or *Listeria* : between 40 and 50% of interested labs.

As previously noticed, interprofessional labs have other wishes. They are mainly interested in the enumeration of butyric spores (81%), and less in *Salmonella* and / or *Listeria* detection: between 60 and 70%, and even less in the enumeration of coliforms or of *E. coli* (50%).

↳ **In conclusion**, many thanks for answering the questionnaire. It will certainly not be easy to reply to so many different wishes, but we will try and we are hoping to hear from you when our first « microbiological » reference samples will be available.

FORTHCOMING EVENTS

➲ REMINDER

		CONTACT
24 – 28 july 2000 **BALTIMORE, MD, USA** Rencontres annuelles ADSA – ASAS *American Dairy Science Association / American Society of Animal Science*	2000 ADSA/ASAS Joint meeting 111 North Dunlap Avenue Savoy IL 61874 USA	tel : 1/217.3563182 fax : 1/217.3984119 e-mail : adsa@assochq.org asas@assochq.org
6 – 9 august 2000 **ATLANTA, USA** 87th International Association for Food Protection annual meeting *formerly* IAMFES *International Association of Milk, Food and Environmental Sanitarians*	**IAMFES** 6200 Aurora Ave Suite 200W DES MOINES IA 50322-2863 USA	Tel : +1/515.2763344 Fax : +1/515.276.8655 e-mail : iamfes@iamfes.org http://www.iamfes.org
10 – 14 september 2000 **PHILADELPHIA, USA** 114th AOAC International annual meeting *Association of Official Analytical Chemists*	**IDF** Secrétariat 41, square Vergote B-1030 BRUXELLES BELGIUM	Tel : 32/2.733.1690 Fax : 32/.2.733.04.13 e-mail : info@fil-idf.org http://www.fil-idf.org
16 – 20 september 2000 **DRESDEN, GERMANY** 84th IDF annual sessions	**IDF**	

➲ OTHER EVENTS

3 – 9 SEPTEMBER 2000 **LISBONNE, PORTUGAL** Euroanalysis XI	**Dra Cristina OLIVEIRA** Dpt Quimica & Bioquimica Fac. Ciencas. Univ. Lisboa Edificio C1 – Piso 5 P-1700 LISBOA, PORTUGAL	Fax : +351 1 3909352
18 – 20 SEPTEMBER 2000 **AVEIRO, PORTUGAL** 5th international conference on applications of magnetic resonance in Food Science	Fundaçao Joao Jacinto de Magalhaes Edificio I Campus Universitario de Santiago 3810-193 AVEIRO, PORTUGAL	tel : +352 234 380901 Fax : +352 324 370084 e-mail : mrfood2000@dq.ua.pt http://www.dq.ua.pt/icmrafs
20 – 22 SEPTEMBER 2000 **PRAGUE, CZECH REP.** Chemical reactions in food IV European Conference on new knowledge on chemical reactions during processing and storage of foods	**Pr Dr Jiri DAVIDEK** Dpt Food Chemistry & Analysis Institute of Chemical Technology Technicka 1905 166 28 PRAGUE 6, CZECH REP.	Fax : +420 2 3119990 e-mail : Jiri.Davidek@vscht.cz
10 OCTOBER 2000 **BRUSSELS, BELGIUM** CEN seminar on food safety and European standardization	**CEN** *Comité Européen de Normalisation* *European Standardization Committee*	e-mail : kristien.vaningelgem@cenorm.be
16 – 20 OCTOBER 2000 **ANTWERP, BELGIUM** CAC 2000. 7th international conference on chemometrics in analytical chemistry	CAC 2000 secretariat University of Antwerp Dpt Chemistry Universiteitsplein 1 B-2610 ANTWERP-WILKIJK, BELGIUM	Fax : +32 3 820 2376 e-mail : cac2000@uia.ua.ac.be http://sch-www.uia.ac.be/cac2000
19 – 20 OCTOBER 2000 **LONDON, UNITED KINGDOM** Food safety in Europe. Challenge and opportunities		http://www.foodsafetyeurope.com/body_home.html

INTERESTING NEW STANDARDS

IDF STANDARDS

IDF 170A :1999. .Milk and milk products. Enumeration of presumptive *Escherichia coli.* Part 1: Most probable number technique / Part 2: Most probable number technique using 4-methylumbelliferyl-beta-D-glucuronide (MUG) / Part 3: Colony-count technique at 44°C using membranes

This version replaces the 1994 provisional version.

INTERNATIONAL (and french) STANDARDS

ISO 9622 Whole milk. Determination of milkfat, protein and lactose content Guidance on the operation of mid-infrared instruments
This text is almost equivalent to IDF standard 141B :1996

ISO 1211. Milk. Determination of fat content. Gravimetric reference method

ISO 11056 Sensory analysis. Methodology. Magnitude estimation method

EUROPEAN (and french) STANDARDS

EN ISO 1737. Evaporated milk and sweetened condensed milk. Determination of fat content. Gravimetric reference method

EN ISO 7328. Milk-based edible ices and ice mixes. Determination of fat content. Gravimetric reference method

There are mostly editing differences between these two standards and the previous versions, issued 1985. However an important methodological difference is that now the operator **may use pentane instead of light petroleum**.

LIST OF BIBLIOGRAPHIC REFERENCES

You will find enclosed the list of references that we found in our litterature survey over the past months and that we decided to put into our data base on dairy analytical techniques. Should you be interested in any of these references, please contact us.

➢ **We also noticed**

A special issue of « Lait » New applications of membrane technology in the dairy industry , containing the papers issued during the Saint-Malo symposium in June 1999. (Lait, 2000, Vol. 80, N. 1)

The influence of amyl alcohol in the Gerber method.

(Abstract of the lecture given by Mr TROSSAT of CECALAIT at CECALAIT's annual general meeting in June 1999.)

The Gerber acidobutyrometric method has been widely used as a routine method for the determination of fat in milk in most dairy laboratories and in most countries. Indeed, it is easier and cheaper than the reference Röse-Gottlieb method. However, the accuracy of the routine method has always been carefully examined by the standardization bodies, because it is well known that these two methods are not strictly equivalent. Moreover, some discrepancies between results coming from labs in different countries have been observed, depending on the origin of one reagent : amyl alcohol (see box at the end).

In France, CECALAIT, AFNOR, official laboratories (DGCCRF) and interprofessional laboratories worked together. Firstly, to solve the problem of differences, due to different amyl alcohols by a better definition of its composition. Secondly, the work was aimed at proposing an ideal reagent to ensure equivalent results between the Gerber and the Röse-Gottlieb methods, in a fat range as wide as possible.

↳ INFLUENCE OF ISOAMYL ALCOHOL

In the Gerber method, amyl alcohol should make easier fat separation in the butyrometer. However, a few years ago, some laboratories noticed differences in fat results depending on the origin of the alcohol. The differences were observed among french laboratories and also, more importantly, between french and foreign laboratories.

It was suggested that some alcohols might not comply with the specifications of the annex of V 04-210 french standard, describing the Gerber method (cf box). So, official laboratories analysed 7 usual alcohols, from 7 different suppliers and concluded that each of them complied with the standard. However, analysis by gas chromatography of the ratio of the two isomers present in the mixture showed that the proportion of 3-methyl-1-butanol varied from 70% to 100%.

Meanwhile, the examination of the results obtained from 1991 to 1996, in Cecalait's proficiency studies showed a discrepancy between Gerber and Röse-Gottlieb results. It was about –0,3 g at the time, for mean fat ranges (it is much smaller now), but was not constant for all fat levels.

So, in 1997, an interlaboratory study was organised to check the influence of the composition of amyl alcohol on the accuracy of the Gerber method.

It involved 10 laboratories analysing in duplicate, by the Gerber method, 10 samples with fat ranges from 15 to 50 g/l. They used 3 different alcohols with following isomer ratios :

- 3-methyl-1-butanol : 83% and 2-methyl-1-butanol : 17% (M3B1/M2B1 83/17)
- 3-methyl-1-butanol : 91% and 2-methyl-1-butanol : 9% (M3B1/M2B1 91/9)
- 100% 3-methyl-1-butanol (M3B1/M2B1 100/0)

The means of the results were compared to the results obtained at the same time on the same samples in an interlaboratory study using the Röse-Gottlieb reference method.

Table 5, page 11 in « La Lettre de CECALAIT » shows the results and the significative influence of the isomer ratio of amyl alcohol.

↳ TOWARDS AN « IDEAL » ISOMER RATIO …

Treating the results of this first interlaboratory study allowed the calculation of an « ideal » isomer ratio, for equivalent results between the Gerber and the Röse-Gottlieb method, when fat ranges from 15 to 50 g/l.

The 94/6 M3B1/M2B1 was thus calculated and validated through an interlaboratory trial.

Performed in 1998, it involved 28 interprofessional laboratories analysing, by the Gerber method with that type of alcohol, 10 samples with fat ranging from 15 to 50 g/l. The results were compared to those obtained at the same time by 21 laboratories analysing the same samples by the Röse-Gottlieb method : they showed a mean bias of about 0.05 g/l.

An assesment meeting in May 1999 highlighted the following points:

- amending the composition of amyl alcohol improves the adjustment of the two methods,

- Since 1996, the discrepancy between the two methods has reduced greatly, maybe because a change occured in the manufacture of the alcohol at the time. According to suppliers, the isomer ratio is generally about 95/5 of M3B1/M2B1 since the beginning of 1997.

- the French interprofession wishes the two methods to be equivalent in the mid fat ranges, between 30 and 50 g/l. This is indeed the most representative range for milk payment and trade, corresponding to the great majority of individual or herd french milks.

Therefore new calculations were necessary and finally it is the ratio **M3B1/M2B1 91/9** which has been proposed.

↳ TOWARDS THE STANDARD'S REVISION

Since then, AFNOR has almost completed a new version of V 04-210 standard, which will :

- specify the precise composition of the amyl alcohol, ie a mixture of the M3B1/M2B1 isomers in the proportion **91%/9%**, with an uncertainty of ± 2% for each part of the ratio. (This would have a maximum incidence of ± 0.06 g/l on the final result).

- specify the application domain for fat ranging from 30 to 50 g/l. In this common domain, both methods are equivalent. The standard will point out the differences which might be observed outside of this domain. In this case, other (internal) validation studies are highly desirable.

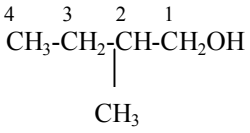
Suppliers should also be kept in touch in order to ensure that the manufactured alcohols comply with the isomer ratio specified in the standard. Eventually, this ratio will be put on the certificate of compliance of the reagent.

This new version of standard V 04-210 should be issued at the end of this year.

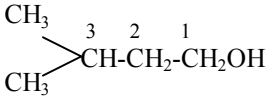
The amyl alcohol used in the Gerber method

This reagent, $C_5H_{12}O$, is actually, a mixture of two isomers.

2-methyl-1-butanol, or amyl alcohol
or *dl-sec*-butylcarbinol



3-methyl-1-butanol, or isoamyl alcohol
or isopentyl alcohol
or isobutylcarbinol



Some specifications of amyl alcohol, according to the annex of V 04-210 standard

- volumic mass between 0.808 and 0.818 g/ml
- colourless
- composed of at least 98%, in volume, of the mixture of following primary alcohols :
  - 3-methyl-1-butanol
  - 2-methyl-1-butanol
- without secondary alcohol.

For abbreviations and bibliography, please see page 12 in La Lettre de CECALAIT