CENTRE D'EXPERTISE ET DE CONTROLE DES ANALYSES LAITIÈRES

2006

2nd quarter

N°57

CECALAIT'S NEWSLETTER

LIFE AT CECALAIT

CECALAIT'S ANNUAL GENERAL MEETING

The AGM was held on 14th June at the "Maison du Lait" in Paris.

Firstly, the 2005 balance sheet was draw up and the auditor certified the ledgers.
Secondly, CECALAIT's activities and the principal events that have arisen since the last AGM were presented, amongst them:

- the advances in accreditation,
- the operations in external communication, particularly concerning the layout of the English search engines on CECALAIT's website and the operations of punctual communication on the use of some SRMs,

The afternoon was dedicated to the presentations of the following subjects:

- Evolution of the standard concerning method validation
  M. FEINBERG INRA

- Presentation of the new European directive on hygiene
  Mrs VION DGAL

- The complementarity of using proficiency tests and SRMs in an analytical laboratory
  M. TROSSAT CECALAIT

Unfortunately, as at last year's AGM, we registered a relatively low participation. We hope that more members will be available next year. Indeed, during the 2007 AGM, the representatives of the different laboratories group will be re-elected.

ACCREDITATION OF CECALAIT FOR THE ORGANISATION OF PROFICIENCY TESTS AND THE REALISATION OF TESTS IN MICROBIOLOGY !

Recently, **COFRAC** accredited CECALAIT for its activity as a proficiency test organiser according to the **LAB-CIL REF 02**. This accreditation covers the following proficiency tests in chemistry: **raw milk**, **homogenised milk**, **lipolysis**, **cream**; and in microbiology: **total flora**.

Moreover, **COFRAC**, according to the **NF EN ISO/CEI 17025** standard, has just acknowledged the competence of CECALAIT's microbiology laboratory for the realisation of the following 3 tests: **micro-organisms at 30°C**, ***Escherichia coli*** and **coagulase positive *Staphylococcus***.

EVALUATION OF FOODLAB®

Foodlab is an analyser, developed by CDR (Italia) and marketed by Grosseron, for analysis of food matrices. It permits, amongst other things, the determination of various parameters in milk and dairy products using specific kits. On a technical level, it is composed of an incubation unit (12 thermostatic wells at 37°C) and a reading unit (3 spectrophotometric wells operating at 3 wavelengths in the visible region).

The tests, realised at CECALAIT from February to September 2005, were performed on the "ammonia" and "alkaline phosphatase (ALP)" tests on milk.

For each test, the results are presented in two distinct parts:
- part A: results issued from calibration realised with a standard, whose values were obtained according to the reference method,
- part B: results recalculated according to the calibration method recommended by the constructor (affectation of theoretical values to standards).

For each evaluation, the Foodlab and reference values are the mean of two repetitions obtained respectively by the instrumental and reference methods (only the duplicates satisfying repeatability conditions of the methods were kept).

EVALUATION OF THE "ALKALINE PHOSPHATASE" (ALP) TEST

The objectives of these tests were:
- on a quantitative level, to evaluate the repeatability of the method and the relation with the reference method NF EN ISO 11816 (1), and
- on a qualitative level, to evaluate the connection between the results obtained by both methods in terms of positive/negative results in relation to a given threshold (0,350 Unit/Litre (U/L), threshold recommended by the DGAL note (2)).

Quantitative study

Sample description

Two sets of samples were used for these tests:
- set 1: 13 samples of milk from a mixture of heat treated and raw milk, to obtain a range of 0.1 to 1% of raw milk (5 samples of mixtures of heat treated milk / raw milk and 8 samples of mixtures of pasteurised milk / raw milk),
- set 2: 10 samples of milk from mixtures of pasteurised and raw milk, to obtain a range of 0.1 to 1% of raw milk.

Methods

These tests were performed according to both methods:
- the reference method, in accordance with the NF EN ISO 11816 standard (1) using Fluorophos® test (3), and
- the instrumental method, in conformity with the constructor's procedure using the ALP kit provided by Grosseron. Its principle is the hydrolysis of p-nitrophenylphosphate with alkaline phosphatase, which generates, in an alkaline medium, a chromogenic compound. Its intensity, measured at 405 nm, is directly proportional to the phosphatasic activity of the sample. This relation is established and can be modified at any time, by calibration of the analyser.

Results

➪ Part A

➡ Calibration

The analyser was calibrated using 3 points (pasteurised milk with 0%, 0.1% and 0.2% of raw milk), with reference values obtained using the reference method.
A specific calibration was performed for each batch of kits.
(Set 1: K = 14.68 and Q = -14.99 ; Set 2: K = 15.73 and Q = -0.13) K and Q : slope and intercept of the analyser calibration graph.

➡ Evaluation of repeatability

For each set, the repeatability was calculated according to ISO 5725 standard (4) using indicators:

- Sr (standard deviation of repeatability) = $\sqrt{(\Sigma\ w_i^2 / 2n)}$ with w_i: deviation between duplicates, and n: number of duplicates
- r (maximal deviation between duplicates) = 2,77.Sr

Set 1: Sr = 0.012 U/L and r = 0.033 U/L for an average level of 2.61 U/L

Set 2: Sr = 0.016 U/L and r = 0.044 for an average level of 3.30 U/L

➡ Evaluation of the relation with the reference method

For each set, the relation was evaluated by performing a linear regression between the instrumental and reference results. Figures 1a and 2a illustrate the results obtained.

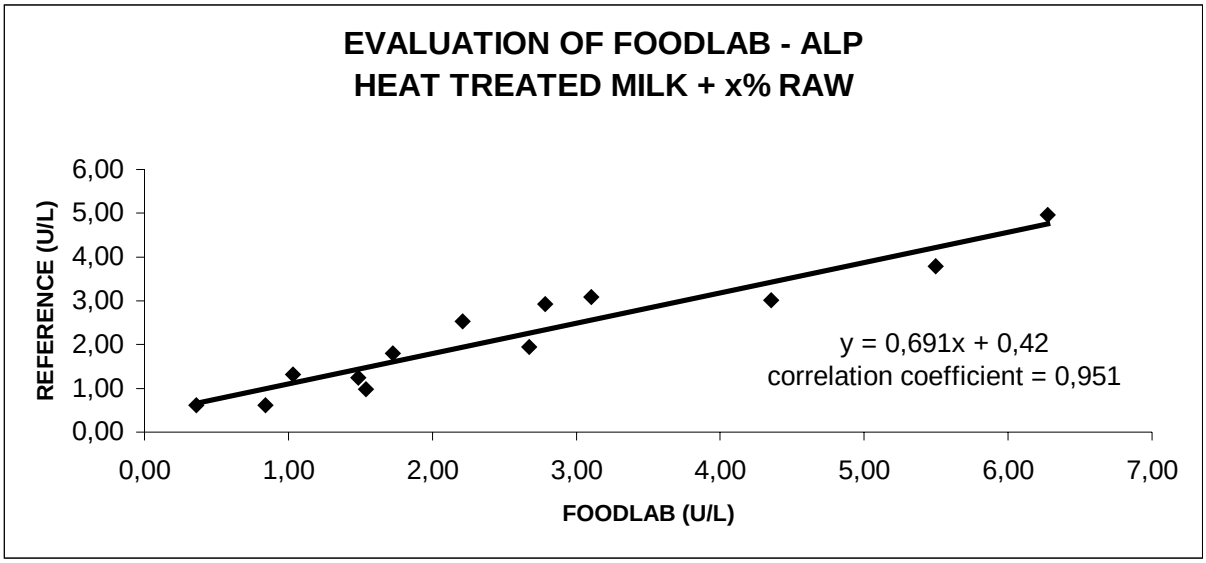

figure 1a: Relation between the instrumental results and the reference values concerning the ALP criterion (set 1)

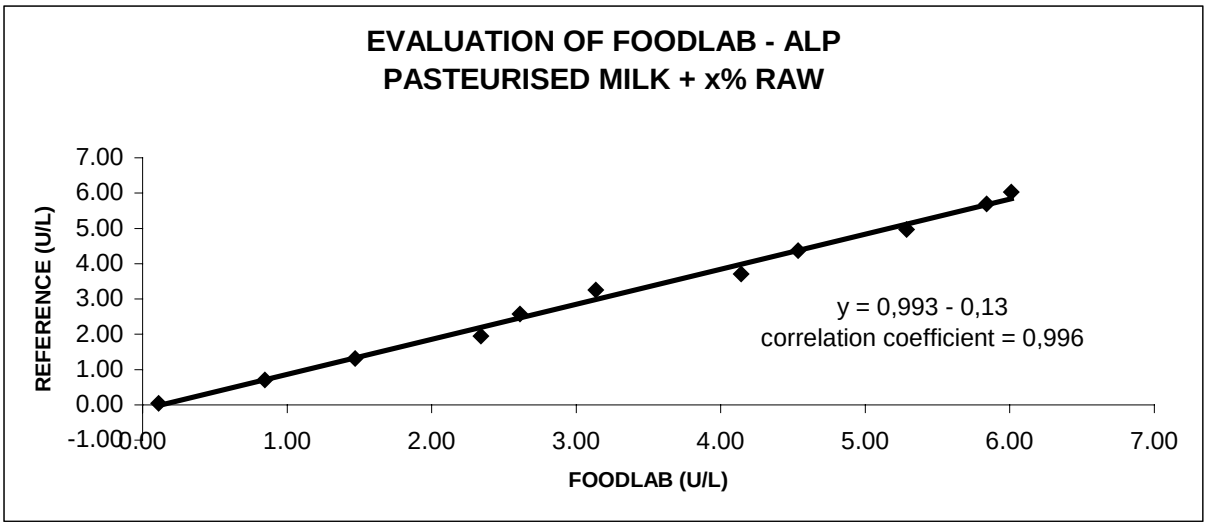

figure 2a: Relation between the instrumental results and the reference values concerning the ALP criterion (set 2)

For set 1, the relation between the methods is linear to about 6 U/L, and the correlation coefficient (0.951) is near to 1.
For set 2, this relation is optimal as the slope and the intercept are near to 1 and 0 respectively, and the correlation coefficient near to 1.

➪ Part B

➡ Calibration

The values were recalculated according to the calibration recommended by the constructor: 3 points (pasteurised milk with 0%, 0.1% and 0.2% of raw milk) with reference values (0.01, 1 et 2 U/L respectively).
(Set 1: K = 24.70 and Q = -25.30 ; Set 2: K = 24.83 and Q = -0.29)

➡ Evaluation of repeatability

For each set, the repeatability was calculated according to ISO 5725 standard (4) using the indicators described in part A.

Set 1: Sr = 0.034 U/L and r = 0.094 U/L for an average level of 4.31 U/L

Set 2: Sr = 0.041 U/L and r = 0.113 for an average level of 5.13 U/L

➡ Evaluation of the relation with the reference method

For each set, the relation was evaluated by performing a linear regression between the instrumental and reference results. Figures 1b and 2b illustrate the results obtained.

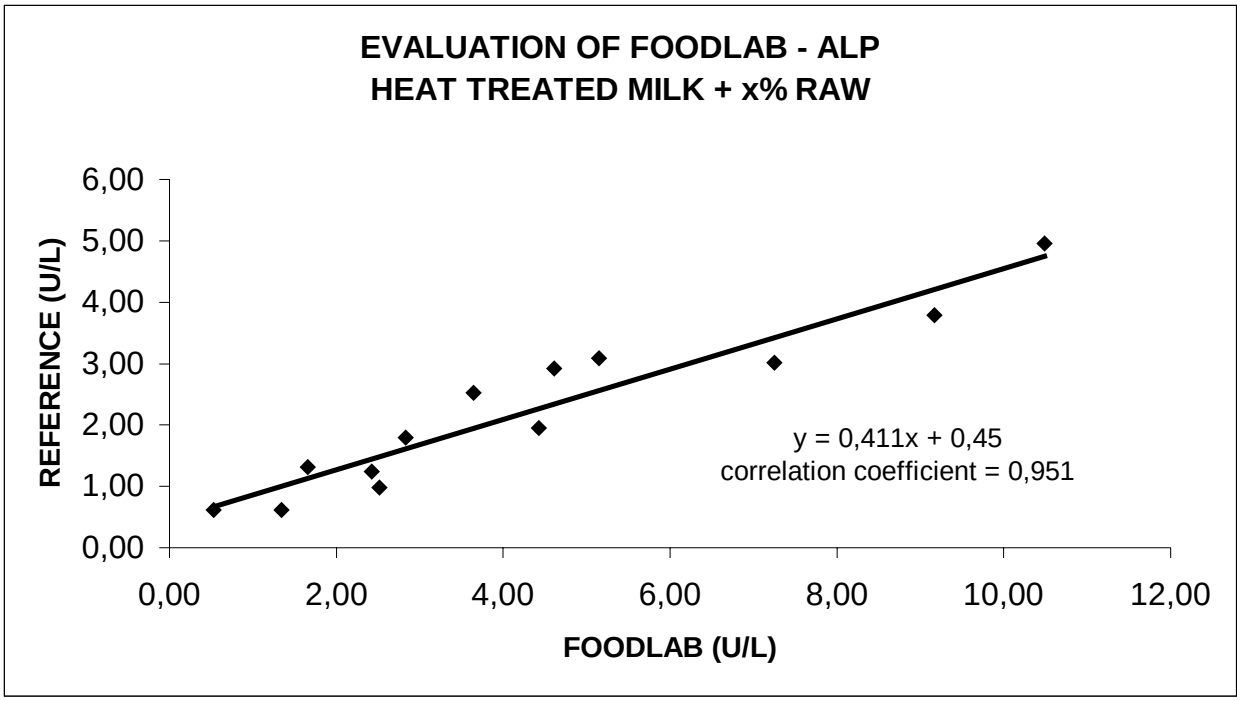

figure 1b : Relation between the instrumental results and the reference values concerning the ALP criterion (set 1)

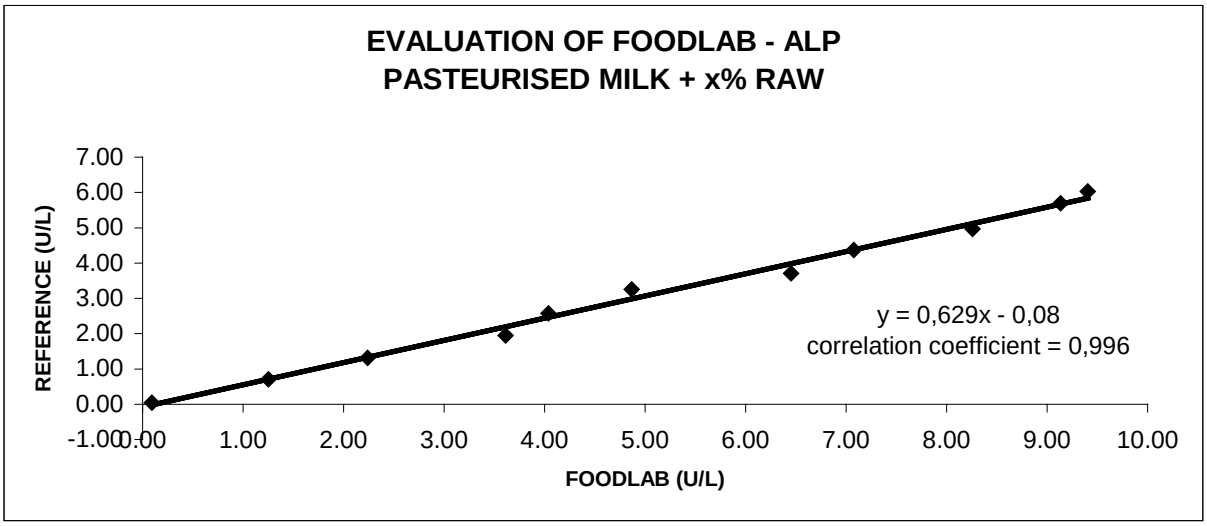

figure 2b : Relation between the instrumental results and the reference values concerning the ALP criterion (set 2)

For both sets, the relation is linear with a good correlation, but the results deviate more from the reference values than the results obtained in part A.

Qualitative study

Description of samples and methods

As previously, the tests were realised with 2 sets:
Set 1: -5 samples of raw bulk milk from the Franche-Comté region

-5 samples of full fat UHT and pasteurised drinking milk.

Set 2: -6 samples of UHT (full fat and semi-skimmed) and pasteurised drinking milk

-11 samples of milk from a mixture of pasteurised and raw milk constituted to obtain a range from 0.01 to 0.1 % of raw milk.

The methods used were the same as in the quantitative study.

Results

➪ Part A

➡ Calibration

As in the quantitative study, specific calibrations were realised for each batch of kits (Set 1: K=14.68 and Q = -14,99 ; Set 2 : K = 8,85 and Q = -1,18).

➡ Samples

The result is negative if the value obtained is below 0.350 U/L and positive above.

For all the raw bulk milk and drinking milk samples, the results obtained by the instrumental and reference methods were equal.
For the mixtures of milk, the detection of a positive result (> 0.350 U/L) was obtained from 0.01% of raw milk for the Foodlab, against 0.03% for the reference method.

Graphical examination shows that the results can be improved by a correction of the calibration using standards between 0 and 0.1% of raw milk (according to the equation Y = 0.7093 - 0.1062**.** The results are then in total concordance with the reference values.

➪ Part B

➡ Calibration

As in the quantitative study, specific calibrations for each batch of kits were realised with theoretical values provided by the constructor (Set 1: K = 24.70 and Q = -25.30 ; Set 2: K = 8.31 and Q = -0,20).

➡ Samples

The result is negative if the value obtained is below 0.350 U/L and positive above.

The results of part A and part B are concordant.

Conclusion

From a practical point of view, the Foodlab "ALP" test is simple.
On a quantitative level, the repeatability is satisfactory in relation to the specifications of the reference method, which fixes the maximal deviation between duplicates at 0.062 U/L, for an average level of 0.500 U/L. A good correlation can be noted between the instrumental and reference results, and the relation between both methods is linear. However, the results are closer to the reference values after calibration with values obtained using the reference method.

On a qualitative level, for all the calibration modes, the results are very satisfactory. Indeed, they are perfectly in accordance with the results obtained by the reference method on raw, mixture and drinking milk samples. The detection threshold of raw milk is near to the reference method threshold. The results are more accurate when they are obtained from a calibration realised in relation to the reference values.

However, concerning the calibrations, the results show that:

- K and Q parameters vary according to the variations in kit manufacture, which implies systematic calibration for each set.
- the reference values are very important according to the preparations. It is important to realise the calibration with reference values determined by analysis rather than with fixed values assigned in accordance with the percentage of raw milk.
- a calibration between 0 and 0.1% of raw milk will permit to improve the precision of results around 0.35 U/L.

EVALUATION OF THE « AMMONIA » TEST

The objective of the tests was to evaluate the repeatability and the accuracy of the results obtained by the analyser in comparison with the standardised reference method NF V 04-217 (5).

Description of the samples

The tests were performed with 2 sets:

- set 1: 22 samples of milk including:
  -12 samples of raw bulk milk from the Franche-Comté region, and
  - 10 samples of raw milk with added ammonia, to obtain a range of about 5 to 80 ppm. These samples were prepared by dilution, with an aqueous solution at 25%, of a milk enriched with ammonia.

- set 2: 27 samples of milk including:
  - 8 samples of raw milk with added ammonia, to obtain a range of about 5 to 70 ppm. These samples were prepared, according to the recommendations of the constructor, by dilution, with ammonium sulphate, of a milk enriched in ammonia, and
  - 19 samples of raw bulk milk from the Franche-Comté region.

Methods

The tests were performed according to both methods:
- the reference method, in accordance with the NF V 04-217 (5) standard using the urea/ammonia test developed by Boehringer Manheim (6).
- the instrumental method, in conformity with the constructor's procedure using the ammonia kit provided by Grosseron. The principle is the formation of an ammonium-phenolic by-product complex, in alkaline medium, generating a chromogenic compound. The measured intensity is

directly proportional to the quantity of ammonia in the sample. This relation is established and can be modified at any time by calibration of the analyser.

Results

➪ Part A

➡ Calibration

For set 1, the initial calibration was used without any modification (K = 33.97 ; Q = 0.,59).

For set 2, two specific calibrations were realised for each batch of reagents.
1st calibration: K = 46.38 and Q = 8.78
2nd calibration: K = 27.39 and Q = 10.93

➡ Evaluation of repeatability

Repeatability was calculated according to ISO 5735 standard (4) on both sets of samples and to the method described above.

Set 1 (9 samples of supplemented milk):
Sr = 0.75 ppm and r = 2.06 ppm for an average level of 17.31 ppm
Set 2 (19 samples of raw milk):
Sr = 1.23 ppm and r = 3.41 ppm for an average level of 10 ppm

➡ Evaluation of accuracy

Supplemented samples

Accuracy was evaluated by a linear regression between the instrumental and reference results (mean of duplicates) on the samples of set 1 (figure 3a) and set 2 (figure 4a).

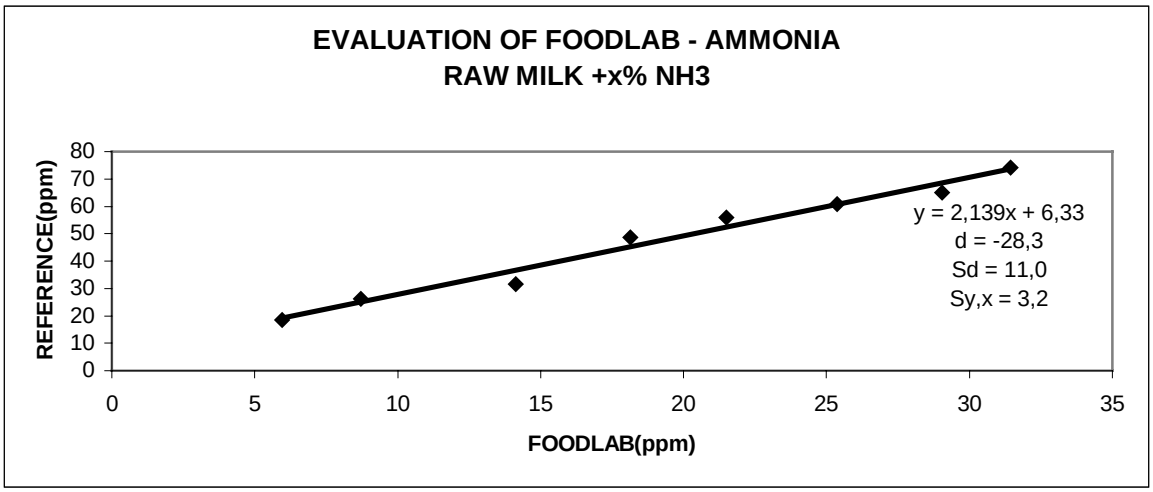

figure 3a : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (supplemented milk set 1)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

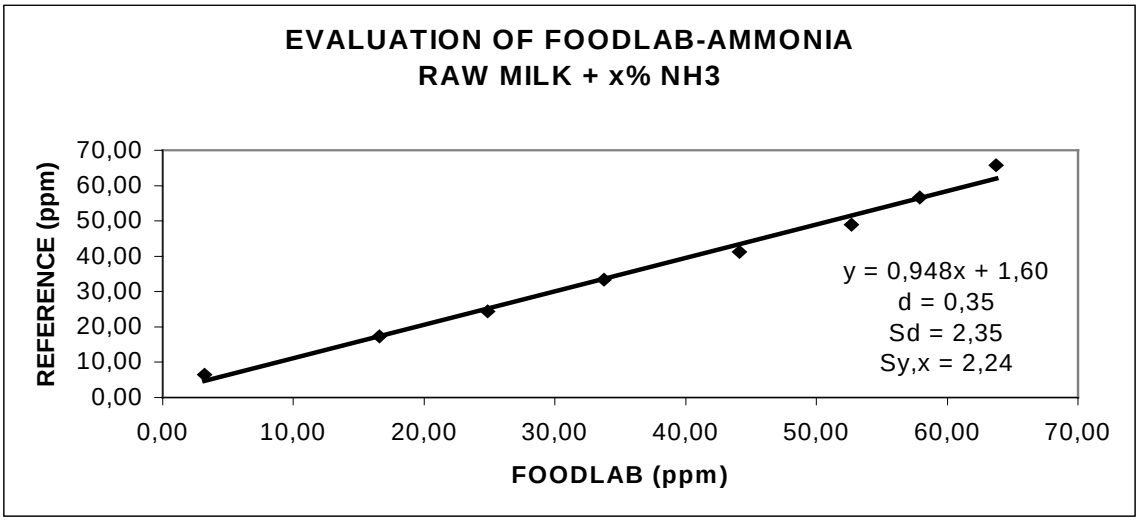

figure 4a : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (supplemented milk set 2)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

For set 1, in spite of a slope (2.139) and an intercept (about 6 ppm) deviating respectively from 1 and 0, and a mean deviation of –28, the relation between the methods is linear between about 18 and 70 ppm. Indeed, the residual standard deviation obtained in this range is about 3 ppm.
For set 2, the results are concordant as the mean deviation is close 0, the deviation and residual standard deviation are close and low (about 2 ppm).
The differences in accuracy observed between set 1 and set 2 are due to the calibration mode.

Raw milk samples

Accuracy was evaluated by calculating the mean deviation (d) and the deviation of standard deviation (Sd) between the instrumental and reference results (mean of duplicates).

- 1st calibration (7 raw milk samples):
d = -6.9 ppm and Sd = 1.52 ppm.

- 2nd calibration (12 raw milk samples):
d = 1.16 ppm and Sd = 2.17 ppm.

There is an important mean deviation for the results obtained with the 1st calibration, whereas the results obtained from the 2nd calibration are clearly closer to the reference.

➪ PartB

For set 2, the results were recalculated with the calibration installed by the constructor (K=33.97; Q=0.59)
The Foodlab and reference values are from the mean of both repetitions obtained by the instrumental and reference methods (calculated under repeatability conditions).

➡ Evaluation of repeatability

Repeatability was calculated according to ISO 5735 standard (4) on both sets of samples and to the method described above.

Set 1 (9 supplemented samples):
Sr = 0.75 ppm and r = 2.06 ppm for an average level of 17.31 ppm
Set 2 (19 raw milk samples) :
Sr = 1.86 ppm and r = 5.15 ppm for an average level of 2.23 ppm

➡ Evaluation of accuracy

Supplemented samples

Accuracy was evaluated by establishing a linear regression between the instrumental and reference results (mean of duplicates) of the milk samples of set 1 (figure 3b) and set 2 (figure 4b).

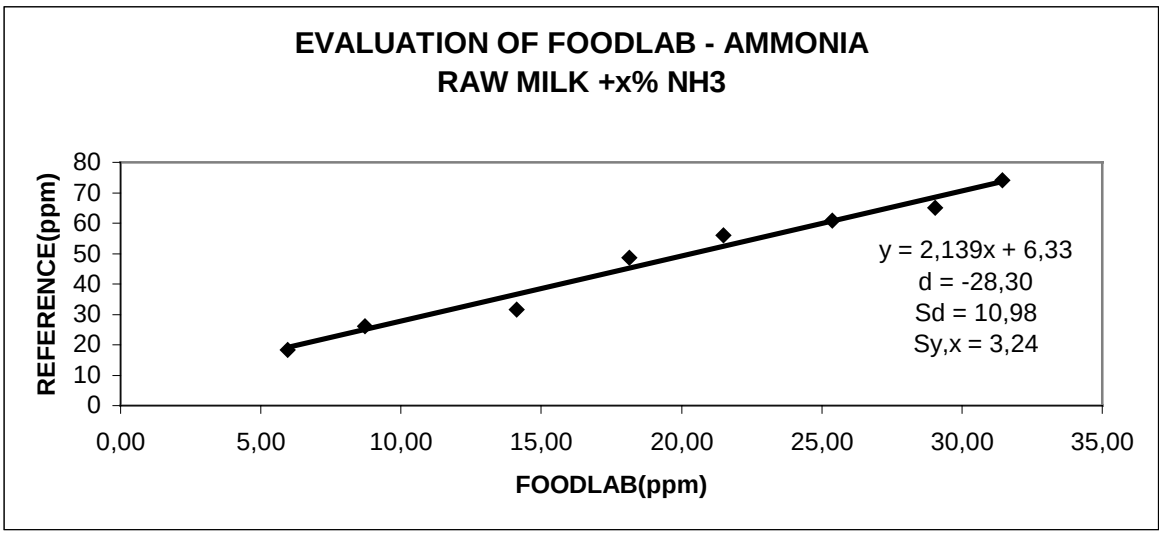

figure 3b : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (set 1)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

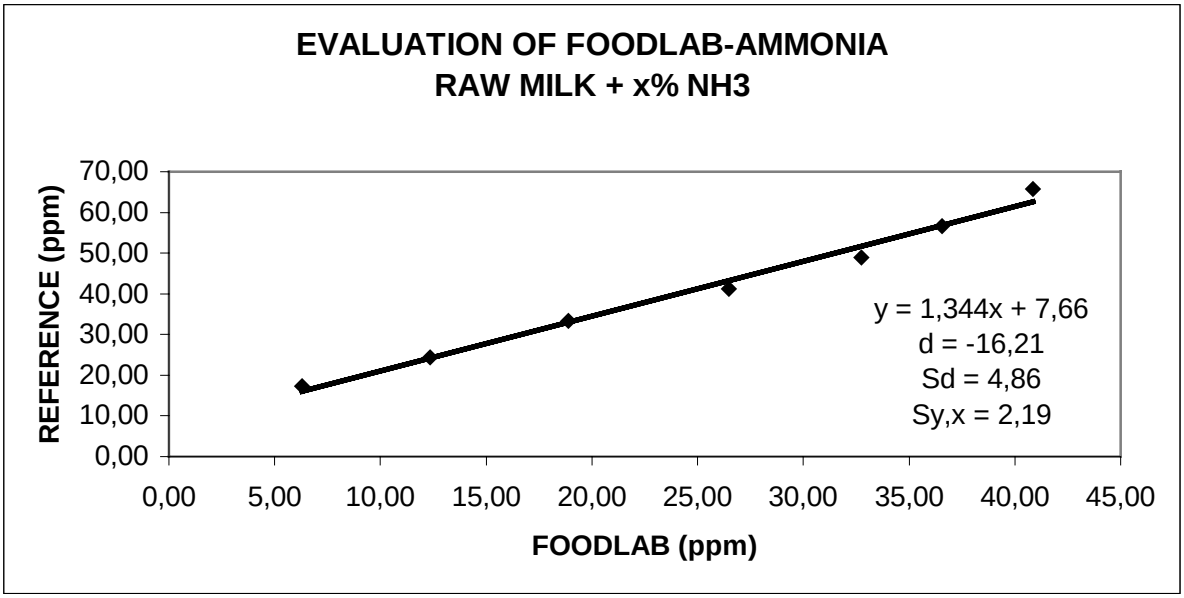

figure 4b : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (set 2)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

As with set 1, the results of set 2 deviate from the reference values.

Raw milk samples

Accuracy was evaluated by calculating the mean deviation (d) and the deviation of standard deviation (Sd) between the instrumental and reference results (mean of duplicates).

- 1st calibration (7 raw milk samples):
d = -14.12 ppm and Sd = 1.45 ppm.

- 2nd calibration (12 raw milk samples):
d = -8.70ppm and Sd = 2.57ppm.

The results deviate more from the reference values than in part A.

Conclusion

As with the "ALP" test, the "ammonia" test is simple to use. However, concerning the latter, some difficulties to obtain coherent repeatability results were observed. Several results then had to be eliminated and the corresponding tests done again. These high deviations between duplicates did not come from the analyser but from the kits.

Repeatability is satisfactory in relation to the reference method specifications, which fix a maximal deviation of 2.8 ppm between duplicates.

For part A, the evaluation of accuracy, on the supplemented samples (set 2), shows a good correlation between the methods and permits to envisage a precision in estimation lower than 5 ppm over the range of 18 to 70 ppm (5% risk).
Concerning the raw milk samples, the mean deviation obtained with the 1st calibration is high. Indeed, it is about 7 ppm for an average level of 12 ppm. This deviation is most certainly due to an inappropriate calibration.
The results of the samples obtained with the 2nd calibration also represent an average level of 12 ppm. The mean deviation and the deviation of standard deviation (respectively about 1 and 2 ppm) permit a precision in estimation corresponding to about 5 ppm (5% risk).

For part B, the results deviate more from the reference values.

Thus, as for the ALP test, concerning the calibrations:
- K and Q parameters vary according to variations in kit manufacture, which implies systematic calibration for each batch.
- the reference values of standards are very different according to the preparations. It is therefore, very important to calibrate with reference values determined by analysis, rather than with fixed theoretical values assigned in relation to the quantity of ammonium sulphate added.

Thanks to RADIOMETER for the loan of Fluorophos ® material

REFERENCES :

(1) NF EN ISO 11816-1 standard: June 2000 « Determination of alkaline phosphatase activity – Part 1: Fluorimetric method for milk and milk-based drinks» 7 pages. AFNOR editions
(2) DGAL note « Avis relatif aux méthodes et normes utilisables pour vérifier la conformité aux critères microbiologiques des laits de consommation et produits à base de laits lors de leur mise sur le marché » JORF 251 du 27/10/2004.
(3) Fluorophos® test system – Advanced instruments inc.
(4) NF EN ISO 5725-2: 1994 «Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method». AFNOR editions
(5) Norme NF V 04-217: 1992 « Lait et produits laitiers -Détermination de la teneur en ammonia et en urée – méthode enzymatique » 9 pages. AFNOR editions
(6) Test Urea/Ammonia – « UV method for the determination of urea and ammonia in foodstuffs … » Ref 10 542 946 035 Boehringer Mannheim/R-biopharm

RESULTS OF THE QUESTIONNAIRES CONCERNING THE SETTING UP OF NEW SERVICES AT CECALAIT

In December 2005, you received two questionnaires concerning the setting up of new SRMs, one about butter and the other about dried milk. A lot of laboratories replied and we are very grateful to you. Here are the results obtained.

SRMs on butter

151 questionnaires were sent to laboratories and 26,5 % replied. Among these answers, 80 % seem to be concerned. You will find in the tables below their wishes concerning these samples:

DESIRED PRODUCTS	Number of laboratories
Sweet butter 82% Fat	**30**
Sweet butter 41% Fat	5
Half-salted butter 82% Fat	**25**
Half-salted butter 41% Fat	5
Other:	
Sweet butter 60% Fat	*1*
Sweet butter 25% Fat	*1*

SAMPLE QUANTITY	Number of laboratories
125g	9
250g	**18**
Other:	
350g	*1*

FREQUENCY OF USE	Number of laboratories
1 per week	1
2 per month	1
1 per month	8
1 per quarter	11
on request	10

DESIRED CRITERIA	Number interested
Moisture	**32**
Fat	**19**
Non fat dry matter	**24**
Chloride	**23**
Other:	
Fat acidity	*9*
pH	*6*
Water distribution	*1*
Phosphatase	*1*
Peroxide value	*1*
Methylic ethers GC	*1*
% of butyric acid	*1*
Enumeration of Staphylococcus aureus	*1*

SRMs on dried milk

65 questionnaires were sent to laboratories and 36,9 % replied. Among these answers, 83,3 % seem to be concerned. You will find in the tables below their wishes concerning these samples:

DESIRED PRODUCTS	Number of laboratories
Whole dried milk	10
Semi-skimmed dried milk	3
Skimmed dried milk	15
Other:	
Dried whey	*3*
*Low fat whey and re-fattened **ou** Low fat and re-fattened whey*	*2*
Milk proteins >75% proteins	*2*
Skimmed dried goat's whey	*1*
Infant formula 1st et 2nd stage	*1*
Re-fattened milk	*1*
Buttermilk	*1*

SAMPLE QUANTITY	Number of laboratories
35g	8
50g	12

DESIRED CRITERIA	Number of laboratories
Moisture	**17**
Fat	14
Nitrogen	**17**
Lactose	4
Other:	
Minerals	*3*
Acidity	*1*
NH_3	*1*
WPN-Ca	*1*

SAMPLE QUANTITY	Number of laboratories
35g	8
50g	12

FREQUENCY OF USE	Number of laboratories
1 per week	0
2 per month	3
1 per month	5
1 per quarter	6
on request	7

Many thanks for informing us about your needs and wishes. Homogeneity and stability tests are currently being carried out on both these products. Following this, we will be able to propose these new SRMs on butter and dried milk in our 2007 catalogue of services.

IN THE PRESS – ON THE WEB

Classification in alphabetical order of keywords

PATHOGENS / DAIRY PRODUCTS

Test speeds up pathogen detection for dairy sector

http://www.foodproductiondaily.com/news/printNewsBis.asp?id=67627

► Biotrace International develops a test allowing a sped up pathogen detection in dairy products.

NEW EU STANDARDS AND REGULATIONS

Classification is established in alphabetical order of the first keyword

ORIGIN / FOODSTUFFS

O.J.E.U. L 93, 31st March 2006 – Council Regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs
http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2006/l_093/l_09320060331en00120025.pdf

FLAVOURING SUBSTANCES / FOODSTUFFS

O.J.E.U. L 91, 29th March 2006 – Commission Decision of 27 March 2006 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs
http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2006/l_091/l_09120060329en00480048.pdf

REFERENCE LABORATORIES

O.J.E.U. L 136, 24th May 2006 – Commission Regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community reference laboratories
http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2006/l_136/l_136200605224en00030008.pdf

ORGANIC PRODUCTION / FOODSTUFFS

O.J.E.U. L 137, 25th May 2006 – Commission Regulation (EC) No 780/2006 of 24 May 2006 amending Annex VI to Council Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs
http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2006/l_137/l_13720060525en00090014.pdf

FORTHCOMING EVENTS

Classified in chronological order

DAIRY PRODUCTS

24-27 September 2006 Düsseldorf, Germany	International trade fair dairy products	http://www.intermopro.de
20-23 October 2006 Shanghaï, China	IDF 27th International Congress and World Dairy Summit	http://www.idf2006shcn.com

STANDARDS, DRAFT STANDARDS

Classification in alphabetic order by theme

ISO published standards

CHEESE AND PROCESSED CHEESE PRODUCTS		
CHEESE / PROCESSED CHEESE CITRIC ACID	ISO 2963:2006 (IDF 34) May 2006	CHEESE AND PROCESSED CHEESE PRODUCTS Determination of citric acid content – Enzymatic method
FOOD MICROBIOLOGY		
PATHOGENS / PCR	ISO 20837 : 2006 April 2006	MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFF Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Requirements for sample preparation for qualitative detection
PATHOGENS / PCR	ISO 20838 : 2006 April 2006	MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Requirements for amplification and detection for qualitative methods
MILK AND DAIRY PRODUCTS		
MILK / DAIRY PRODUCTS / PHOSPHATASE	ISO 11816-1 : 2006 (IDF 155-1) April 2006	MILK AND DAIRY PRODUCTS Determination of alkaline phosphatase activity Part 1: Fluorimetric method for milk and milk-based drinks
MILK AND MILK-BASED PRODUCTS		
MILK / MILK BASED PRODUCTS / THERMONUCLEASE	ISO 8870 : 2006 (IDF 83) May 2006	MILK AND MILK-BASED PRODUCTS Detection of thermonuclease produced by coagulase-positive staphylococci
MILK FAT		
MILK FAT / PEROXIDE VALUE	ISO 3976:2006 (IDF 74) March 2006	MILK FAT Determination of peroxide value
MILK PRODUCTS		
MILK PRODUCTS / *LACTOBACILLUS ACIDOPHILUS*	ISO 20128:2006 (IDF 192) May 2006	MILK PRODUCTS Enumeration of presumptive Lactobacillus acidophilus on a selective medium – Colony-count technique at 37°C

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