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.

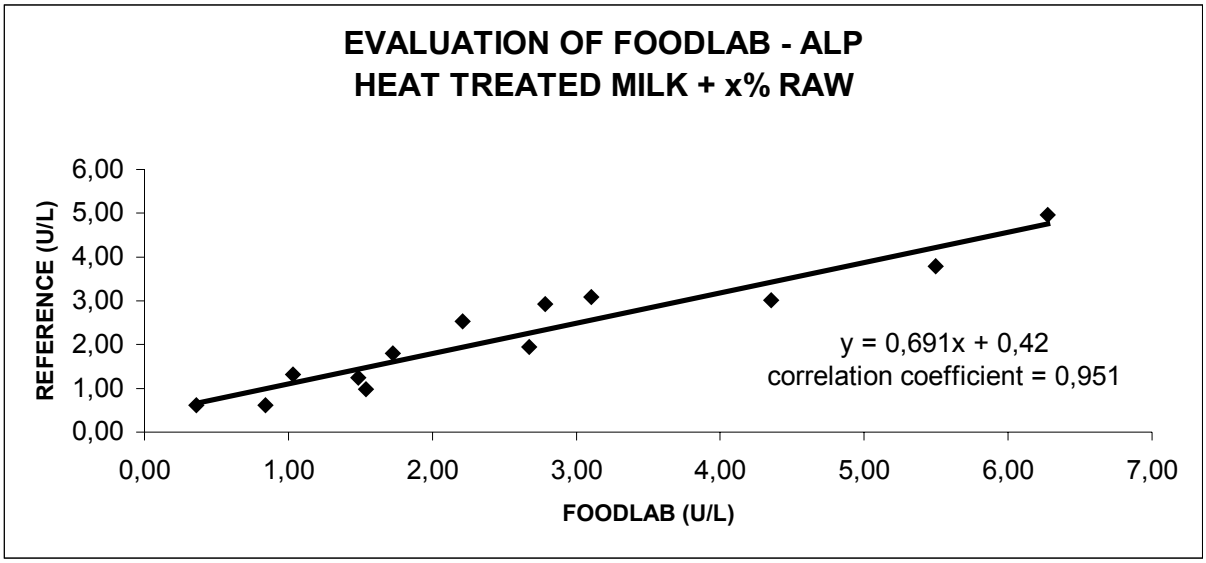

figure 1a: Relation between the instrumental results and the reference values concerning the ALP criterion (set 1)

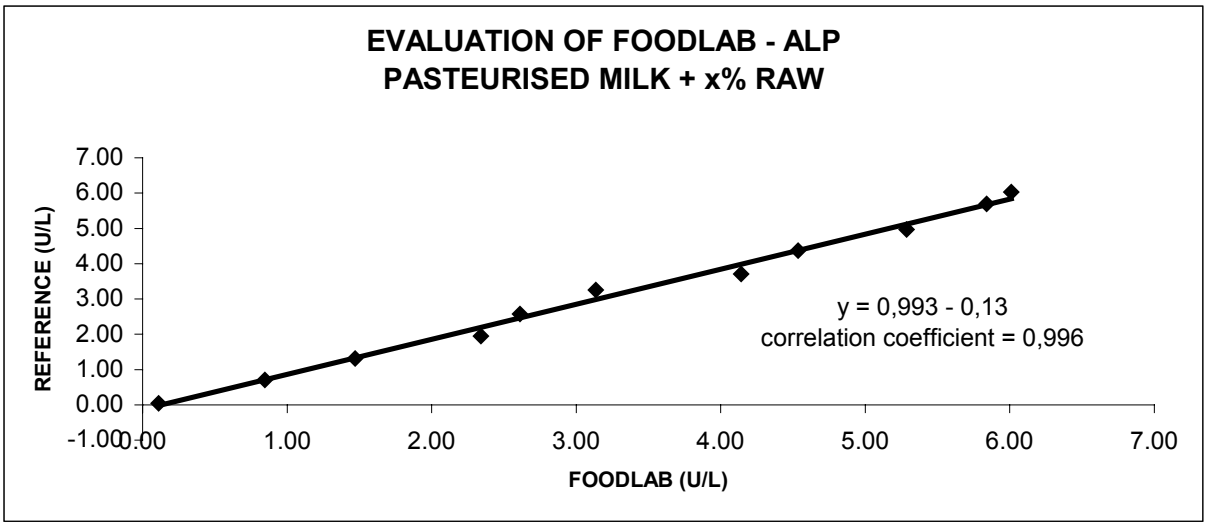

figure 2a: Relation between the instrumental results and the reference values concerning the ALP criterion (set 2)

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.

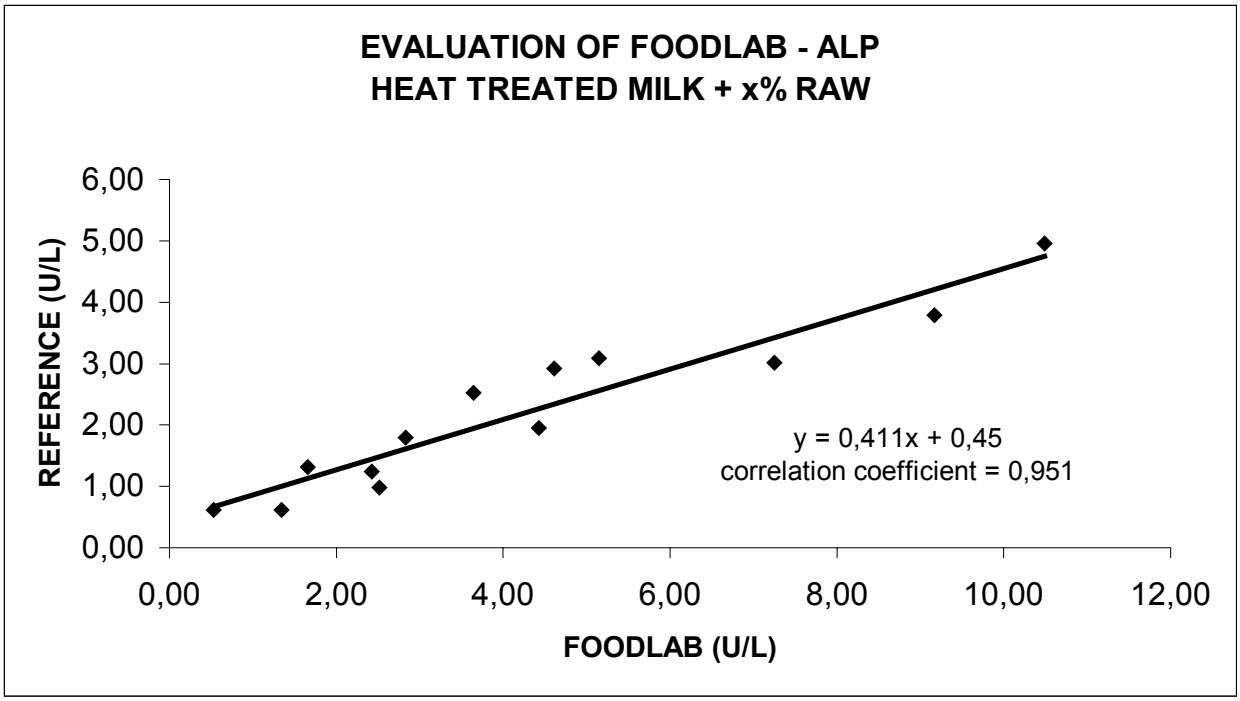

figure 1b : Relation between the instrumental results and the reference values concerning the ALP criterion (set 1)

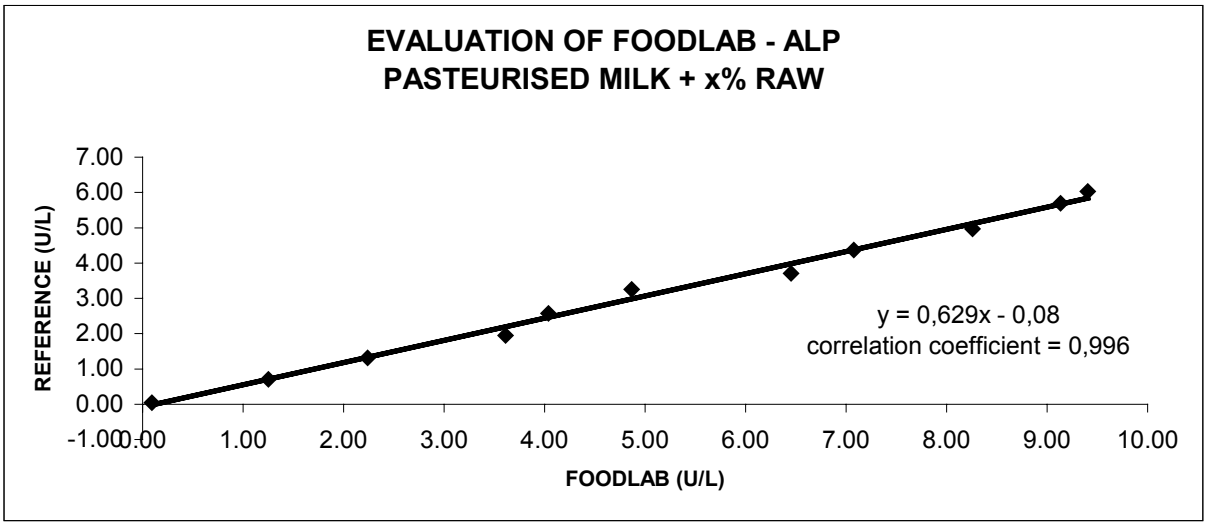

figure 2b : Relation between the instrumental results and the reference values concerning the ALP criterion (set 2)

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).

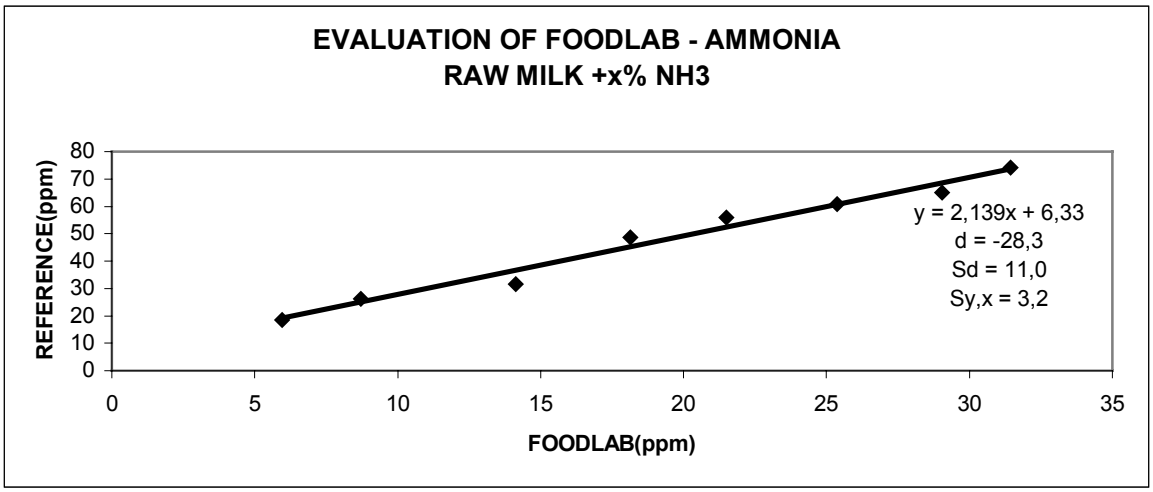

figure 3a : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (supplemented milk set 1)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

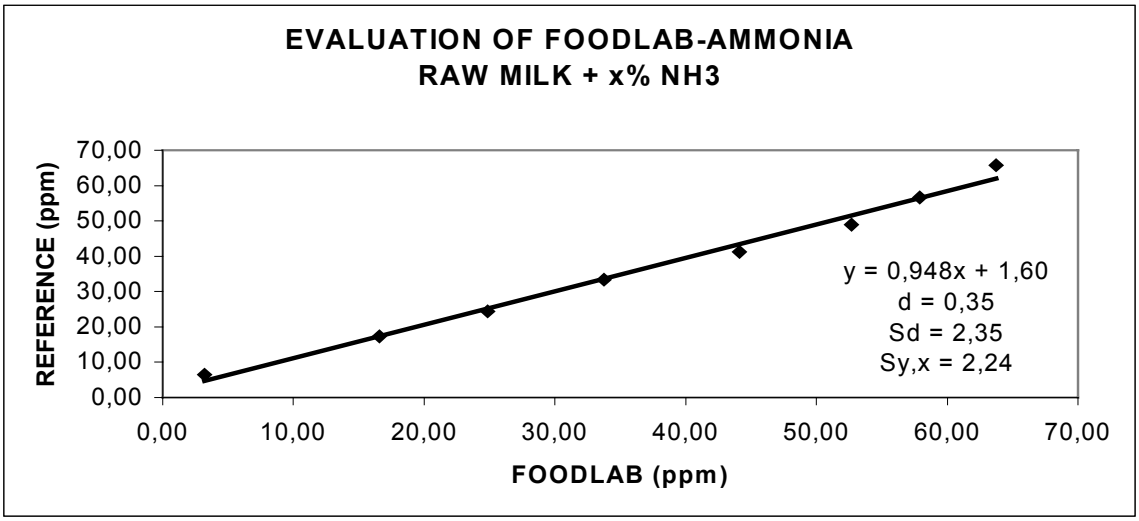

figure 4a : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (supplemented milk set 2)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

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).

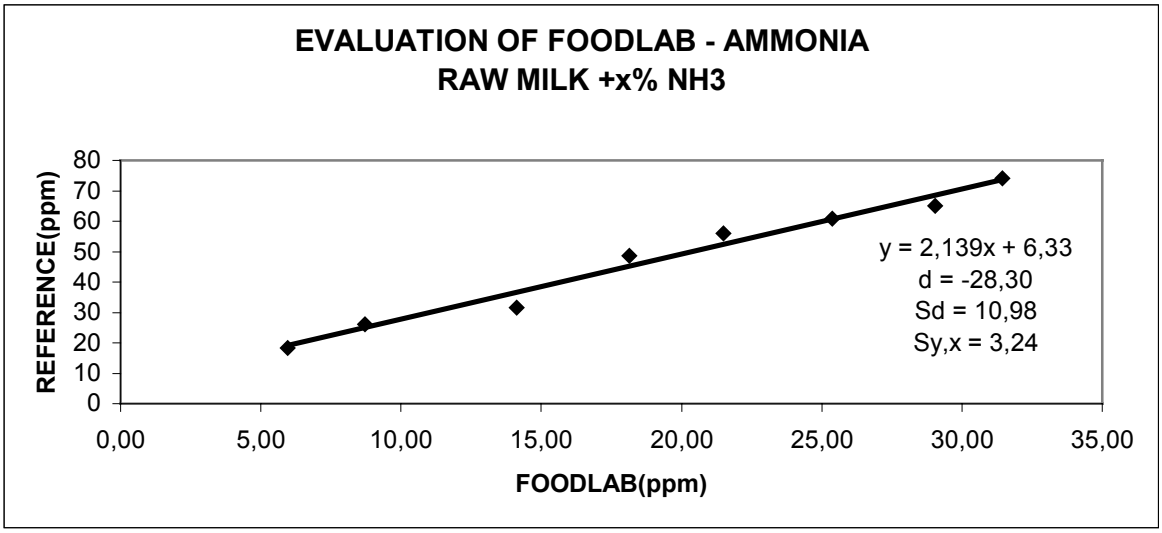

figure 3b : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (set 1)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

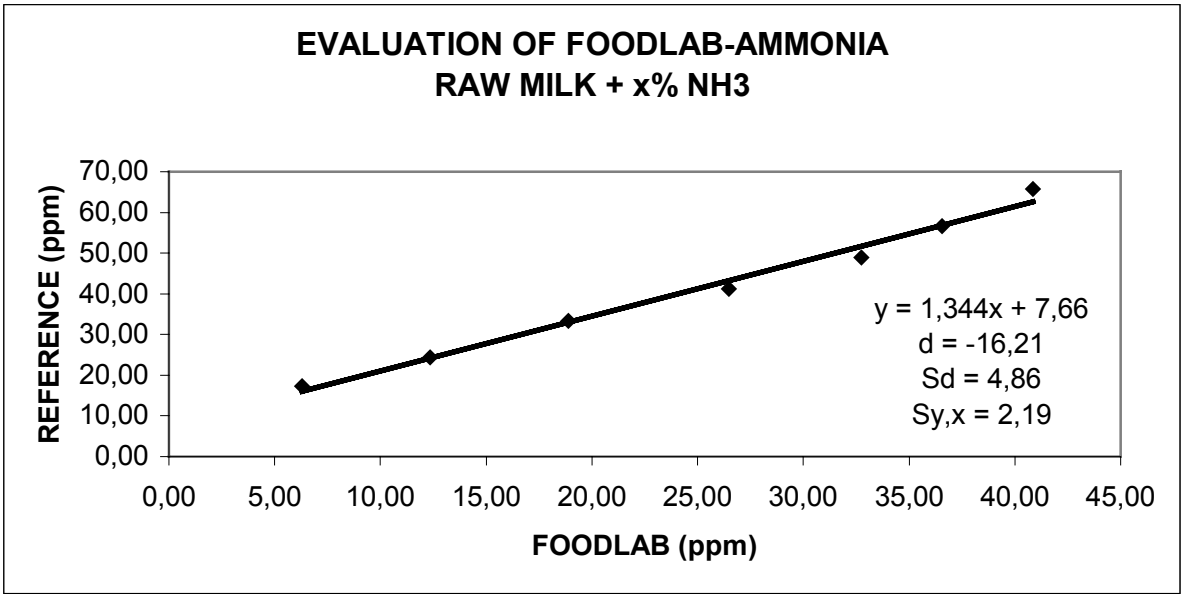

figure 4b : Relation between the instrumental results and the reference values concerning the "ammonia" criterion (set 2)
d and Sd : mean and deviation of standard deviation ; Sy,x : residual standard deviation of linear regression

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