SUMMARY OF LA LETTRE DE CECALAIT, N° 34 (3rd quarter 2000)

(Translation : A. BAPTISTE, Correction : H. LAMPRELL)

Evaluation of the Bentley B 2000 / B

B 2000/B is an automatic MIR (Mid infrared) analyser, developed and marketed by BENTLEY, for analysis of fat, protein and lactose in milk. It can also measure a freezing point equivalent. CECALAIT evaluated its analytical characteristics for 4 months in 1999 (phase I assay, see Lettre de Cecalait, n°33, page 1)

APPARATUS

BENTLEY B2000/B is a dispersive mid infrared spectrophotometer, for the determination of fat, using 2 different filters (filters 1 and 2), protein and lactose in milk. Run by a micro-computer for analyses and calibration, its analysing speed is 450 samples/h.

Mathematically, calibration is done as follows : the instrument measures the energy absorption at specific wavelengths in the mid infrared region. The measure is, first , linearized using a logarithmic algorithm. Then, intercorrection factors are calculated by multiple linear regression (MLR).

TESTS PERFORMED

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
- preservative influence
- 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 20 mn for half a day.

The results show that, for fat (filters 1 and 2) and for protein, the standard relative deviation of reproducibility S_R is always lower than the value inferred by the IDF standard 141B, i.e **S_R < 0.27g/kg or /l**.

❷ CARRY-OVER EFFECT

The carry-over effect was evaluated by analysing, for fat (filters 1 and 2) , protein and lactose, 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 :

$$Tc\ \% = [(S(water\ 1) - S(water\ 2))/ (S(milk\ 2) - S(water\ 2))] \times 100$$

Tc values are in the interval of **0.00 to 0.26%.**

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.

❸ INFLUENCE OF THE PRESERVATIVE

The test was performed on 37 individual cow milks, from two different herds and the results obtained in following cases were compared:

- on raw milk.
- on milk + Bronopol 0.02 %, at 4°C.
- on milk + Bronopol 0.02 %, at 20°C.

Samples were bottled after 4 hours without preservative at 10 to 15°C. For each milk, samples with and without preservative were analysed one after the other, to avoid a drifting effect.

The results do not show any significant difference (at the 1 % limit) between preserved or unpreserved milk or due to the storage temperature. However, for fat determination, higher residual standard deviations were observed when studying the linear regression associated with the storage temperatures; especially when using filter 2, the most sensitive to the quality of milk homogeneization. Later, it will be better to remain vigilant, due to possible fat disruptions through the mechanical homogeneization. With phase II assays analysing more samples in routine, the actual importance of this phenomenon should be better known.

❹ LINEARITY

Linearity was evaluated for each channel by manual analysis, in triplicate, without shaking, of a set of 11 milks with :

- fat ranging from 0 to 85 g/l,
- protein ranging from 5 to 50 g/kg.

The analysis followed first increasing, then decreasing fat and protein levels. Linearity was estimated on raw data, before applying intercorrection factors.

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 for a broader range, for high level milk, for instance Jersey cows’ milk at the end of lactation or ewe’s milk.

❺ REPEATABILITY

Repeatability was evaluated by automatic analysis of 123 individual milk samples and 49 herd milks, preserved with 0.02% bronopol, with fat ranging from 21 to 81 g/l and protein from 24 to 63 g/l.

Each set of 20 samples was analysed in duplicate. The stability of the analyser was checked during the tests.

The values were corrected by linear regression using an orthogonal network of 13 recombined milk samples, following the technique described by O. LERAY in 1989.

The results are given in table 1, page 2, in « La Lettre de CECALAIT » n° 34.

The repeatability values comply with IDF standard 141B specifications, i.e. **Sr = 0.14 g/kg and r = 0.4 g/kg.**

❻ ACCURACY

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

- 110 individual milk samples (same range as in ❺), preserved with 0.02% bronopol, for milk recording purposes,
- 52 herd milks (same range as in ❺), preserved with 0.02% bronopol for milk payment purposes.

The stability of the analyser was checked during the tests. Reference methods used were the official methods for milk payment, ie :

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

The instrument was calibrated using MLR from a set of 13 recombined milk samples (see ❺).

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, where Y is the result of the reference method and X the B 2000/B result.

Tables 2 and 3, page 4, in « La Lettre de CECALAIT » n° 34 show the results on individual and herd milks.

For fat, the mean biases are :

- **+ 0.53 g/l** and **- 0.04 g/l**, for filter 1,
- **+ 0.14 g/l** and **- 0.084 g/l**, for filter 2,

respectively for individual and herd milks.

The slopes are not significatively different from 1.00. The residual standard deviations are :

- **0.84 g/l** and **0.467 g/l,** for filter 1,
- **0.46 g/l** and **0.336 g/l**, for filter 2,

respectively for individual and herd milks. Obviously, using filter 2 reduces the error of the precision of estimation. This is typical of the method.

For protein, the mean biases are :

- **+ 0.04 g/l** and **+ 0.56 g/l,** respectively for individual and herd milks.

The slope is not significatively different from 1.00 for herd milks, but it is for individual milks. The residual standard deviations are :

- **0.552 g/l** and **0.274 g/l,** respectively for individual and herd milks.

However, the deviations observed for slopes and biases remain very small and still comply with users wishes.

In conclusion, for fat and protein, the analytical characteristics of B 2000/B comply with the limits fixed in IDF standard 141, i.e. residual standard deviation of 1.0 g/kg for individual milks and 0.7 g/kg for herd milks.
The mean biases for herd milks are slightly over +/- 0.15 g/kg for protein. It may come from the delay –about a month- between the preparation of the calibration samples and the sampling of herd milks. These differences are then acceptable.

Moreover comparing the herd milk analyses performed on the B 2000/B and on another instrument, already marketed (MS 4000, with B filter for fat) showed equivalent results for the two instruments, i.e :

- for fat, respectively for filter 1 and 2 :
  - mean biases of –0.021 and + 0.013 g/l
  - residual standard deviations of 0.375 and 0.169 g/l,
- for protein, a mean bias of –0.065 g/l and a residual standard deviation of 0.118 g/l.

❼ General conclusion

The analytical characteristics of BENTLEY B 2000/B : instrumental stability, carry-over effect, linearity, repeatability, accuracy, have all been found satisfactory. They all comply with the requirements of milk payment and milk recording purposes.
The procedure authorizing the use of this new material for milk payment purposes (in France, see La Lettre de CECALAIT, n° 33) is still going on. The instrument is now being tested in routine conditions (phase II) for fat and protein and also for FPD.

For abbreviations and bibliography, please see page 5 in La Lettre de CECALAIT n° 34