

## EVALUATION OF THE DELTA INSTRUMENT FTIR 600 LACTOSCOPE INFRARED ANALYSER

The Lactoscope is an infrared spectrophotometer (range  $\mu\text{m}$ ) used for the determination of the principal components in milk. It is manufactured by Delta Instruments (Advanced group, Netherlands) and commercialised in France by Humeau.

This instrument uses a high resolution industrial infrared spectrophotometer based on Fourier transform (FTIR). The complete infrared spectrum (resolution  $8\text{ cm}^{-1}$ ) is collected and recorded for each sample. With a MLR or PLS calibration, various criteria (fat, protein, lactose, dry matter, urea, freezing point...) can be determined.

This apparatus, which is connected to a computer that ensures the signal treatment, can be associated to the "Somoscope" somatic cells counter to constitute the "Combiscope".



The evaluation tests were performed in ACTALIA Cecalait physico-chemistry laboratory (reference and infrared analyses) from February to June 2013. After preliminary tests of stability of the instrument, contamination between samples, linearity and calibration, the repeatability and accuracy were evaluated for fat (equivalent fat filter B), protein, dry matter, urea and freezing point in cow, goat and ewe milk.

A cleaning solution (aqueous solution of Decon<sup>®</sup> at 4 %) and a zero solution (aqueous solution of triton X100<sup>®</sup> at 0,1 %) were necessary for these tests.

The apparatus was configured for a rate of 600 samples per hour and no correction of contamination.

The appreciation criteria of the estimated parameters were taken from ISO 8196-3 / IDF 128-3:2010, or from the CNIEL handbooks concerning the use of infrared apparatus with the context of milk payment and milk control in France (CNIEL PROC IR 06 et CNIEL PROC CR IR 04).

### A. PRELIMINARY TESTS

#### A.1. Evaluation of the stability

The stability was evaluated by the analysis, in automatic mode, of milk every 20 minutes, representing 20 measurement cycles. To evaluate the stability of the instrument, the repeatability and reproducibility were calculated for each analytical criterion and by level..

The values of standard deviation of reproducibility for fat, protein and urea were below to the limits required in ISO 8196-3 / IDF 128-3 (respectively 0.29, 0.58 g/l and 29 mg/l for the median and high values). As no standardised values exist for freezing point, it can be noted that the reproducibility standard deviation values are lower than the limit value of the CNIEL PROC CR IR 04 handbook ( $R = 10\text{ m }^{\circ}\text{C} \rightarrow \text{SR lower than } 3.6\text{ m }^{\circ}\text{C}$ ).

**A.2. Evaluation of contamination between samples**

This criterion was evaluated in automatic analysis mode, by analysing the same cow milk and distilled water according to the sequence: MILK - MILK – WATER – WATER repeated twenty times for fat, protein and freezing point. The evaluation was carried out on 4 levels for fat, protein and protein.

The contamination level was estimated by the formula:  $T_c (\%) = [ (\Sigma(Eau\ 1) - \Sigma(Eau\ 2)) / (\Sigma(Lait\ 2) - \Sigma(Eau2)) ] \times 100$

The contamination rates for fat and protein between successive samples are lower than the maximal limit at 1 % required in the ISO 8196-3 / IDF 128-3 standard and in the CNIEL PROC IR 06 handbook.

The value obtained for freezing point are also lower than the maximal limit at 2 % of the CNIEL PROC IR 06 handbook.

**A.3. Evaluation of linearity**

Volume/volume dilutions were carried out by corrected weighing of density. This corresponds to the principle of quantitative analysis of infrared spectrophotometry and to the French reference measurements.

For fat, a range of 11 milk samples from 0 to 120 g/l was prepared by mixing cream and skimmed milk. Within this range, the Ar/At ratio (Ar and At: amplitude of residues and amplitude of content respectively) is equal to 2.01 %, that corresponds to the limit of 2 % expressed in ISO 8196-3 / IDF 128-3 standard. A linear regression in the range from 0 to about 100 g/l improves the linearity of the instrument (Ar/At ratio equal to 1 %). A Ar/At ratio equal to 0.34 % is obtained with a linear regression in the range from 20 to about 60 g/l, corresponding to the cow milk.

For protein, a range of 11 milk samples from 0 to 80 g/l was prepared by mixing the proteic retentate and filtrate obtained by tangential ultrafiltration (cutoff threshold: 10 KD). Each range was analysed three times. The Ar/At ratio within this range studied is equal to 1 %, which is in conformity with the recommendations of 2 % maximum given in ISO 8196-3 / IDF 128-3 standard. A Ar/At ratio equal to 0.53 % is obtained with a linear regression in the range from 20 to about 40 g/l, corresponding to the cow milk.

The linearity of the instrument is therefore satisfactory for fat (range from 0 to 100 g/l) and protein (range from 0 to 80 g/l).

**A.3. Evaluation of the calibration**

The evaluation of the calibration for fat and protein, initially installed by the manufacturer, was performed with 13 commercial "median" and "high" infrared standard reference materials (SRMs) produced by ACTALIA Cecalait in April 2013. Each sample was analysed in duplicate.

Concerning the median range, the residual standard deviations of the linear regression for fat and protein are closed to the standard deviation of deviations. For fat, an optimisation of the lactose residual interaction enables the reduction of this value (SL3 equal to 0.12 g/l).

Concerning the high range, the residual standard deviations for fat is lower than the standard deviation of deviations. As for the median range, it can be optimised. For protein, the mean bias is high.

To conclude, concerning the median range, the mean bias and the regression slope for fat and protein are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (respectively 0.5 g/l and 1+/-0.05). Concerning the high range, the mean bias (absolute and relative) and the regression slope for fat are in conformity with the recommendations of the ISO 8196-3 / IDF 128-3 standard (respectively 1 g/l, 1.25 % and 1+/-0.05). The mean bias (absolute and relative) for protein are higher than the standardised limits (1 g/l and 1.5 %), probably due to a preliminary defect of calibration on zone milk.

On the other hand, all the residual standard deviations of linear regression obtained for fat and protein are in accordance with the recommendations of the CNIEL PROC IR 06 handbook (respectively lower than 0.25 and 0.15 g/l for the median range and 0.50 and 0.30 g/l for the high range).

## B. EVALUATION OF REPEATABILITY AND ACCURACY

### B.1. The samples

The tests were performed on:

- ◆ for the cow milk: 100 samples of herd milk from the Franche-Comté region and 130 samples of individual milk from 4 farms in the Jura. Bronopol was added to the individual milk samples to give a final concentration of 0.02 %.
- ◆ for the goat milk: 88 samples of herd milk from the Poitou-Charentes region.
- ◆ for the ewe milk: 100 samples of herd milk from the Roquefort sur Souzlon region.

### B.2. Procedure

The repeatability and accuracy of the instrument were evaluated using all the samples for fat, protein and only using the herd cow milk samples for freezing point. In addition; dry matter and urea were respectively measured using herd cow milk samples and individual cow milk samples. The quantitative analyses were performed in automatic analysis mode, in duplicate for each set of 20 samples 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. A control milk was analysed every 20 samples to verify the stability of the analyser. For fat and protein, the evaluation concerns the values obtained after calibration of the instrument with commercial SRMs produced by ACTALIA Cevalait. For freezing point, the instrumental values are from a calibration carried out by the manufacturer.

The following reference methods were used:

- Fat: Gerber acido-butyrometric method according to NF V 04-210: 2000 (single test and then confirmation if more important residues for the individual milk samples) for cow and goat milk samples, and acido-butyrometric method according to NF V 04-155: 2003 (single test) for ewe milk samples,
- Protein: Amido black method according to NF V 04-216: 2011 (test in duplicate),
- Freezing point: thermistor cryoscopic method according to ISO 5764 / IDF 108: 2009 (single test),
- Dry matter: drying method according to ISO 6731 / IDF 21 : 2011 (single test),
- Urea: differential pH-metry method according to ISO 14637 / IDF 195: 2007 (single test).

### B.3. Results

#### B.3.1. Cow milk

##### B.3.1.1. Herd milk

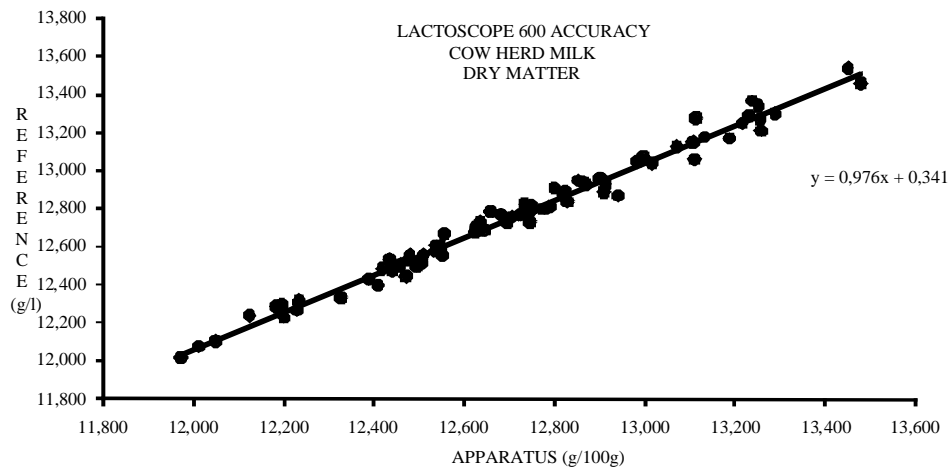
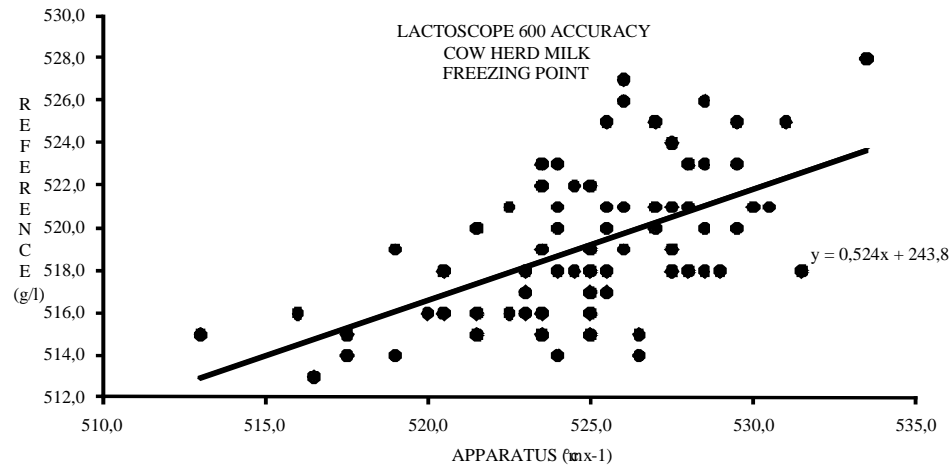
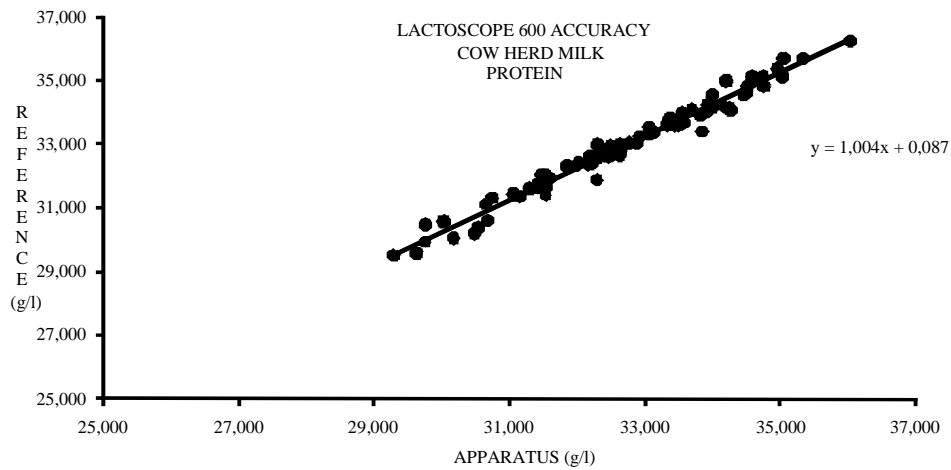
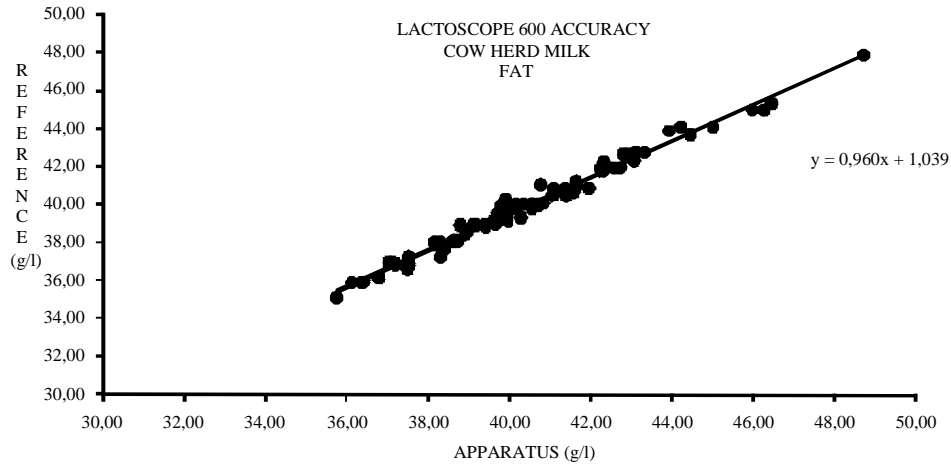
The following tables and figures present the results obtained:

	n	Min	max	M	Sx	Sr	Sr (%)	r
<b>Fat (g/l)</b>	100	35.79	48.75	40.594	2.398	0.055	0.14%	0.150
<b>Protein (g/l)</b>	100	29.32	36.06	32.846	1.502	0.061	0.19%	0.166
<b>Freezing point (m°C x -1)</b>	100	513	534	525.5	3.6	1.1	0.21%	3.1
<b>Dry matter (g/100g)</b>	100	11.98	13.48	12.717	0.327	0.019	0.15%	0.053

Table 1: Lactoscope repeatability criteria for fat, protein, freezing point and dry matter in herd cow milk samples  
*n*: number of results; *min* and *max*: minimum and maximum values; *M* and *Sx*: mean and standard deviation of the results; *Sr* and *Sr* %: absolute an relative standard deviation of repeatability; *r*: maximum deviation of repeatability on 95 % of cases

	n	min	max	Y	Sy	d	Sd	Sy,x	Sy,x (%)
<b>Fat (g/l)</b>	79	35.10	47.80	40.054	2.482	0.547	0.321	0.307	0.76
<b>Protein (g/l)</b>	82	29.54	36.28	33.059	1.543	-0.245	0.230	0.231	0.70
<b>Freezing point (m°C x -1)</b>	84	513	528	519.3	3.4	5.8	3.3	2.8	0.53
<b>Dry matter (g/100g)</b>	79	12.02	13.54	12.762	0.339	-0.048	0.043	0.043	0.34

Table 2: Lactoscope accuracy criteria for fat, protein, freezing point and dry matter in herd cow milk samples  
*n*, *min*, *max*: number of results, minimum and maximum values; *Y*: mean results using the reference method; *Sy*: standard deviation of the results from the reference method; *d*, *Sd*: mean and standard deviation of deviations; *Sy,x*: residual standard deviation.



Figures 1 to 4: Relation between Lactoscope and reference results for fat, protein, freezing point and dry matter in herd cow milk

It can be noted that:

↳ **For fat:** the mean and the standard deviation of deviations are respectively equal to 0.547 and 0.321 g/l. The regression slope (0.960) and the intercept (1.039) are significantly different from respectively 1.00 and zero ( $P = 1\%$ ). The residual standard deviation of regression (0.307 g/l) is in conformity with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 0,72$  g/l).

↳ **For protein:** the mean and the standard deviation of deviations are respectively equal to  $-0.245$  and  $0.230$  g/l. The regression slope (1.004) and the intercept (0.087) are not significantly different from respectively 1.00 and zero ( $P = 5\%$ ). The residual standard deviation of regression (0.231 g/l) is in conformity with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 0,72$  g/l).

↳ **For freezing point:** the mean and the standard deviation of deviations are respectively equal to  $5.8$  and  $3.3$  m °C x  $-1$ . The regression slope (0.524) and the intercept (243.8) are significantly different from respectively 1.00 and zero ( $P = 1\%$ ). The residual standard deviation of regression is equal to  $2.8$  m °C.

↳ **For dry matter:** the mean and the standard deviation of deviations are respectively equal to  $-0.048$  and  $0.043$  g / 100g. The regression slope (0.976) is not significantly different from 1.00 ( $P = 5\%$ ) and the intercept (0.341) is significantly different from zero ( $P = 1\%$ ). The residual standard deviation of regression is equal to  $0.043$  g / 100g.

**B.3.1.2. Individual milk**

The following tables and figures present the results obtained:

	n	Min	max	M	Sx	Sr	Sr (%)	r
<b>Fat (g/l)</b>	130	27.151	53.349	38.650	5.234	0.056	0.15%	0.153
<b>Protein (g/l)</b>	130	26.440	43.461	32.576	3.043	0.066	0.20%	0.179
<b>Urea (mg/l)</b>	130	125.4	483.1	285.4	64.740	14.1	4.94%	38.3

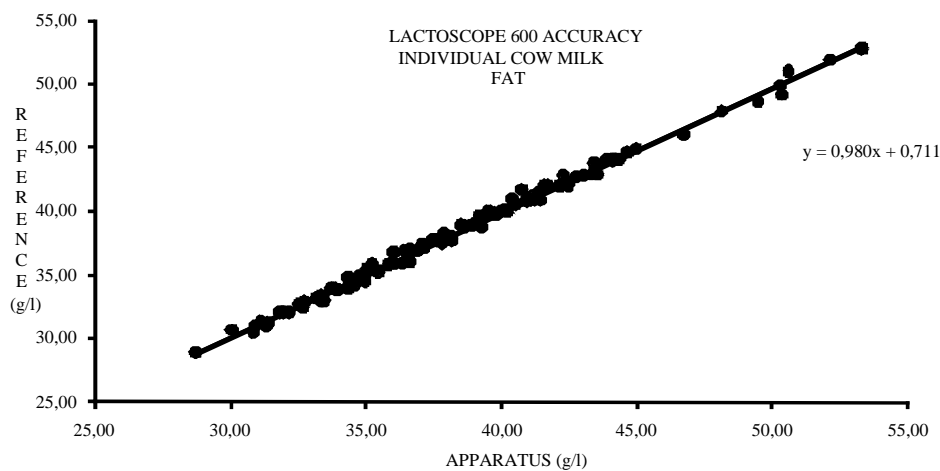
**Table 3:** Lactoscope repeatability criteria for fat, protein and urea in individual cow milk samples

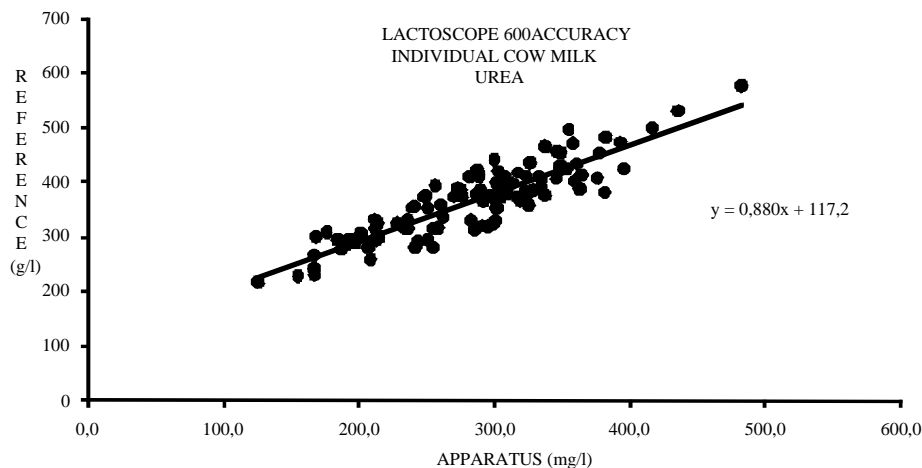
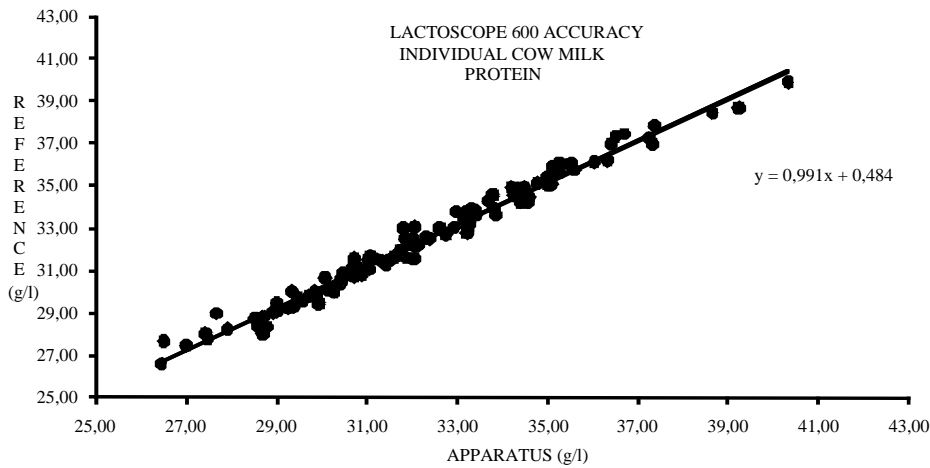
*n:* number of results; *min* and *max:* minimum and maximum values; *M* and *Sx:* mean and standard deviation of the results; *Sr* and *Sr %:* absolute an relative standard deviation of repeatability; *r:* maximum deviation of repeatability on 95 % of cases

	n	min	max	Y	Sy	d	Sd	Sy,x	Sy,x (%)
<b>Fat (g/l)</b>	111	29.00	52.80	38.585	4.937	0.026	0.341	0.328	0.85
<b>Protein (g/l)</b>	111	26.57	39.87	32.585	2.834	-0.205	0.384	0.385	1.19
<b>Urea (mg/l)</b>	109	219	578	369.6	65.0	-82.9	31.3	30.4	10.60

**Table 4:** Lactoscope accuracy criteria for fat, protein and urea in individual cow milk samples

*n, min, max:* number of results, minimum and maximum values; *Y:* mean results using the reference method; *Sy:* standard deviation of the results from the reference method; *d, Sd:* mean and standard deviation of deviations; *Sy,x:* residual standard deviation.





Figures 5 to 7: Relation between Lactoscope and reference results for fat, protein, freezing point and dry matter in individual cow milk

It can be noted that:

↳ **For fat:** the mean and the standard deviation of deviations are respectively equal to 0.026 and 0.341 g/l. The regression slope (0.980) is significantly different from 1.00 ( $P = 1\%$ ) and the intercept (0.711) is not significantly different from zero ( $P = 5\%$ ). The residual standard deviation of regression (0.328 g/l) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 1.03$  g/l).

↳ **For protein:** the mean and the standard deviation of deviations are respectively equal to -0.205 and 0.384 g/l. The regression slope (0.991) and the intercept (0.484) are not significantly different from respectively 1.00 and zero ( $P = 5\%$ ). The residual standard deviation of regression (0.385 g/l) is in conformity with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 1.03$  g/l).

↳ **For urea:** the mean and the standard deviation of deviations are respectively equal to -82.9 and 31.3 mg/l. The regression slope (0.880) is significantly different from 1.00 ( $P = 5\%$ ) and the intercept (117.2) is not significantly different from zero ( $P = 5\%$ ). The residual standard deviation of regression (30.4 mg/l) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 61.8$  mg/l).

**To conclude, for fat and protein in herd and individual cow milk, the standard deviation of repeatability are in accordance with the recommendations of the ISO 8196-3/IDF 128-3 standard and the CNIEL PROC IR 06 handbook ( $S_r \leq 0,14$  g/l). For freezing point, the standard deviation of repeatability obtained is in accordance with the recommendations of the CNIEL PROC CR IR 06 handbook ( $S_r \leq 2$  m °c). For urea, the standard deviation of repeatability is in accordance with the recommendations of the ISO 8196-3/IDF 128-3 standard ( $S_r \leq 14.42$  mg/l). For dry matter, as no standard exists, it can be noted that the standard deviation of repeatability obtained is lower than the limits of the ISO 6731/IDF 21 :2011 standard, which corresponds to the reference method ( $S_r \leq 0,036$  g/l).**

**The results obtained for fat, protein and urea are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3. The residual standard deviation for freezing point (2.8 m °C) and dry matter (0.043 g/100g) enables respectively an accuracy of estimation of +/- 5.6 m °C and +/- 0.086 g/100g.**

**B.3.2. Goat milk**

The following tables and figures present the results obtained:

	n	Min	max	M	Sx	Sr	Sr (%)	r
<b>Fat (g/l)</b>	88	28.91	43.56	34.906	3.108	0.057	0.16%	0.154
<b>Protein (g/l)</b>	88	26.37	36.60	30.694	1.918	0.061	0.20%	0.166
<b>Freezing point (m°C x -1)</b>	88	547	569	557.5	4.3	1.1	0.20%	3.2

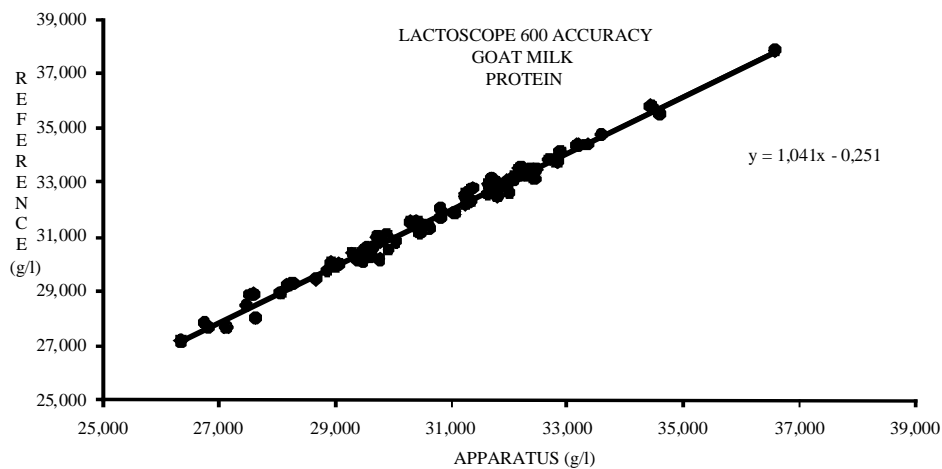
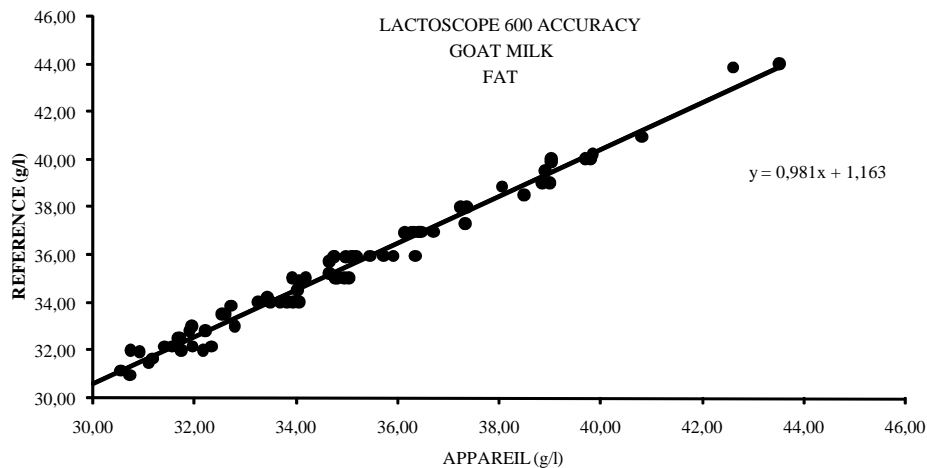
**Table 5:** Lactoscope repeatability criteria for fat, protein and freezing point in goat milk samples  
*n*: number of results; *min* and *max*: minimum and maximum values; *M* and *Sx*: mean and standard deviation of the results; *Sr* and *Sr %*: absolute an relative standard deviation of repeatability; *r*: maximum deviation of repeatability on 95 % of cases

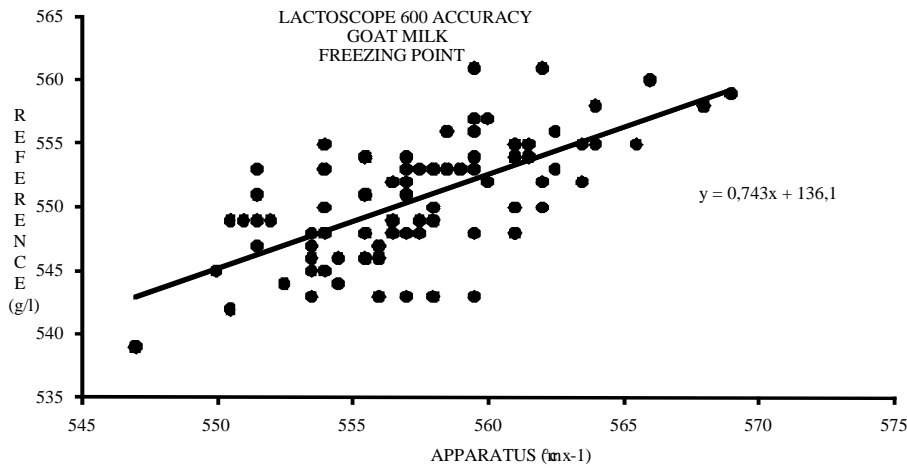
For fat and protein, the standard deviations of repeatability are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard and the CNIEL PROC IR 06 handbook ( $Sr \leq 0.14$  g/l).

For freezing point, the standard deviation of repeatability is in conformity with the recommendations of the CNIEL PROC CR IR 06 handbook ( $Sr \leq 2$  m°C).

	n	min	max	Y	Sy	d	Sd	Sy,x	Sy,x (%)
<b>Fat (g/l)</b>	74	29.90	44.00	35.481	2.961	-0.527	0.384	0.383	1.10
<b>Protein (g/l)</b>	84	27.19	37.86	31.753	2.044	-1.026	0.234	0.222	0.72
<b>Freezing point (m°C x -1)</b>	84	539	561	550.7	4.7	6.8	3.7	3.5	0.63

**Table 6:** Lactoscope accuracy criteria for fat, protein and freezing point in goat milk samples  
*n*, *min*, *max*: number of results, minimum and maximum values; *Y*: mean results using the reference method; *Sy*: standard deviation of the results from the reference method; *d*, *Sd*: mean and standard deviation of deviations; *Sy,x*: residual standard deviation.





Figures 8 to 10: Relation between Lactoscope and reference results for fat, protein and freezing point in goat milk samples

It can be noted that:

↳ **For fat:** the mean and the standard deviation of deviations are equal to  $-0.527$  and  $0.384$  g/l. The regression slope ( $0.981$ ) is not significantly different from  $1.00$  ( $P = 5\%$ ) and the intercept ( $1.163$ ) is significantly different from zero ( $P = 1\%$ ). The residual standard deviation of regression ( $0.383$  g/l) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 0.72$  g/l).

↳ **For protein:** the mean and the standard deviation of deviations are equal to  $-1.026$  and  $0.234$  g/l. The regression slope ( $1.041$ ) is significantly different from  $1$  ( $P = 1\%$ ) and the intercept ( $-0.251$ ) is not significantly different from zero ( $P = 5\%$ ). The residual standard deviation of regression ( $0.222$  g/l) is in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard ( $S_{y,x} \leq 0.72$  g/l).

↳ **For freezing point:** the mean and the standard deviation of deviations are equal to  $6.8$  and  $3.7$  m °C x -1. The regression slope ( $0.743$ ) and the intercept ( $136.1$ ) are significantly respectively different from  $1.00$  and zero ( $P = 1\%$ ). The residual standard deviation of regression is equal to  $3.5$  m °C.

**To conclude, the results obtained for fat and protein are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard. The high mean bias for protein is probably due to a preliminary defect of calibration on goat and zone milk. Despite the absence of standard criteria for freezing point, the residual standard deviation obtained ( $3.5$  m °C) enables an accuracy of estimation of  $\pm 7$  m °C.**

### B.3.3. Ewe milk

The following tables and figures present the results obtained:

	n	Min	max	M	Sx	Sr	Sr (%)	r
<b>Fat (g/l)</b>	100	53.26	85.64	68.111	7.799	0.104	0.15%	0.282
<b>Protein (g/l)</b>	100	45.23	64.20	53.644	4.441	0.110	0.21%	0.309
<b>Freezing point (m°C x -1)</b>	100	538	563	553.5	4.3	1.0	0.18%	2.8

Table 7: Lactoscope repeatability criteria for fat, protein and freezing point in ewe milk samples

*n*: number of results; *min* and *max*: minimum and maximum values; *M* and *Sx*: mean and standard deviation of the results; *Sr* and *Sr %*: absolute and relative standard deviation of repeatability; *r*: maximum deviation of repeatability on 95 % of cases

For fat and protein, the absolute and relative standard deviations of repeatability (*Sr* and *Sr %*) are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard and the CNIEL PROC IR 06 handbook (respectively lower than  $0.29$  g/l and  $0.35\%$ ).

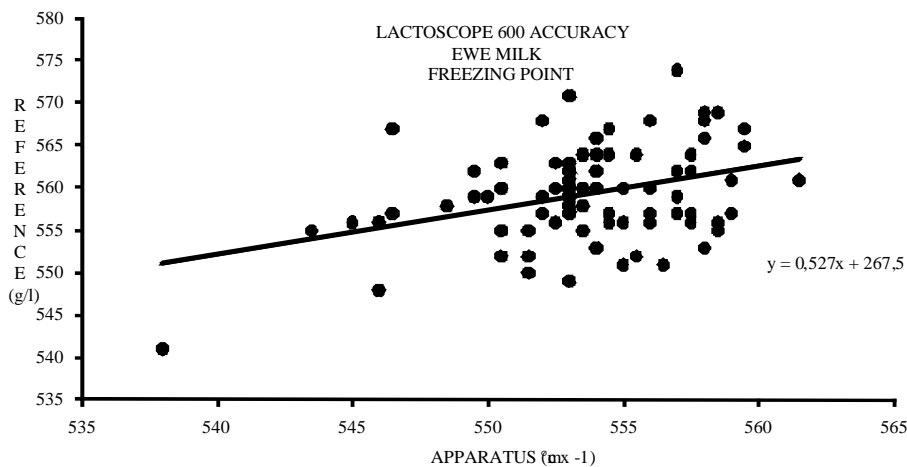
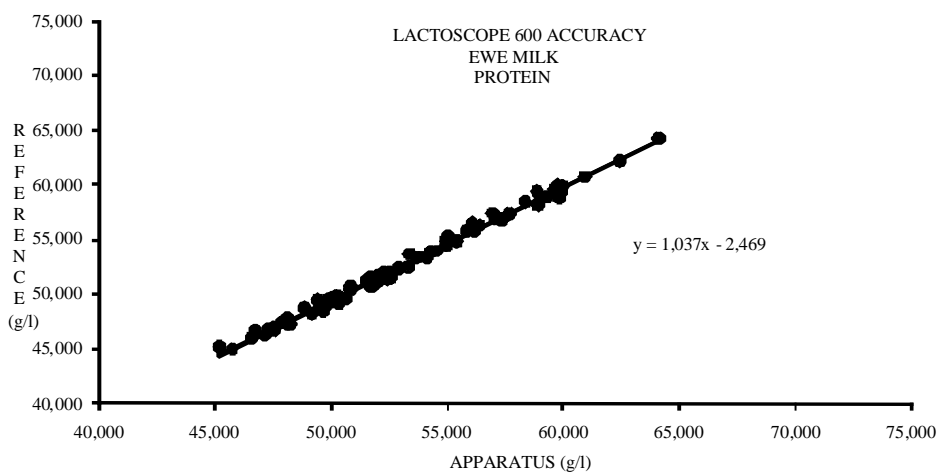
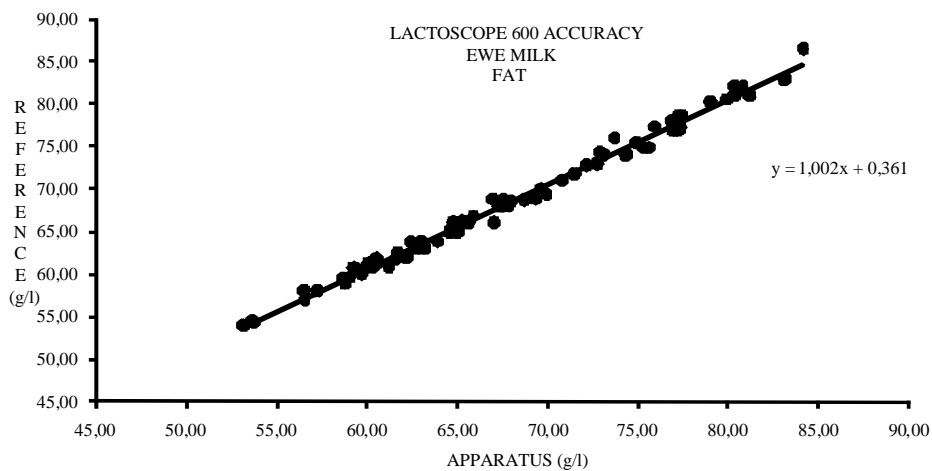
The standard deviation of repeatability obtained for freezing point is in accordance with the recommendations of the CNIEL PROC CR IR 06 handbook ( $S_r \leq 3$  m °C).

	n	min	max	Y	Sy	d	Sd	Sy,x	Sy,x (%)
<b>Fat (g/l)</b>	79	54.00	86.50	68.127	7.699	-0.547	0.665	0.669	0.99
<b>Protein (g/l)</b>	79	45.10	64.28	52.589	4.467	0.459	0.397	0.365	0.69
<b>Freezing point (m°C x -1)</b>	80	541	574	559.4	5.8	-5.7	5.7	5.4	0.97

Table 8: Lactoscope accuracy criteria for fat, protein and freezing point in ewe milk samples

*n*, *min*, *max*: number of results, minimum and maximum values; *Y*: mean results using the reference method; *Sy*: standard deviation of the results from the reference method; *d*, *Sd*: mean and standard deviation of deviations; *Sy,x*: residual standard deviation.





Figures 11 to 13: Relation between Lactoscope and reference results for fat, protein and freezing point in ewe milk samples

It can be noted that:

↳ **For fat:** the mean and the standard deviation of deviations are equal to  $-0.547$  and  $0.665$  g/l. The regression slope (1.002) and the intercept (0.361) are respectively not significantly different from 1.00 and zero ( $P = 5\%$ ). The absolute and relative residual standard deviation of regression (0.69 g/l and 0.99 %) are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (respectively lower than 1.45 g/l and 1.75 %).

↳ **For protein:** the mean and the standard deviation of deviations are equal to  $0.459$  and  $0.397$  g/l. The regression slope (1.037) and the intercept ( $-2.469$ ) are significantly different from 1.00 and zero ( $P = 1$ ). The absolute and relative residual standard deviation of regression (0.365 g/l and 0.69 %) are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard (respectively lower than 1,45 g/l et 2,0 %).

↳ **For freezing point:** the mean and the standard deviation of deviations are equal to  $-5.7$  and  $5.7$  m °C x-1. The regression slope (0.527) and the intercept (267.5) are respectively significantly different from 1.00 and zero ( $P = 1\%$ ). The residual standard deviation of regression is equal to  $5.4$  m °C.

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*To conclude, the results obtained for fat and protein are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3 standard. Despite the absence of standard criteria for freezing point, the residual standard deviation obtained (5.4 m°C) enables an accuracy of estimation equal to +/- 10.8 m °C.*

## CONCLUSION

The results obtained for fat, protein and freezing point are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3: 2010 and/or the CNIEL/IE handbooks concerning the use of infrared apparatus with the context of milk payment and milk control in France (CNIEL PROC IR 06, CNIEL PROC CR IR 04) for the three types of milk (cow, goat and ewe).

Moreover, the results obtained for urea (cow individual milk) are in accordance with the recommendations of the ISO 8196-3 / IDF 128-3: 2010 standard. The results obtained for dry matter (cow herd milk) enable accuracy of estimation lower than 0.1 g/100g.

*According to the evaluation report of the Lactoscope infrared analyser– X. QUERVEL, P. TROSSAT – September 2013*