EVALUATION OF BACTOCOUNT IBC-M

FOR THE ENUMERATION OF SOMATIC CELLS IN MILK

BactoCount IBC-M, manufactured by BENTLEY INSTRUMENTS (US) and distributed in France by Bentley Instruments SARL, is a semi-automatic apparatus for the enumeration of bacteria in raw milk. It uses the principle of flow cytometry with detection by epifluorescent microscopy. It also permits the enumeration of somatic cells in milk.
The sample is collected and manually blended with an incubation reagent made up of a buffered solution of ethydium bromide. This reagent stains the nucleus of the somatic cells.
The mixture is then incubated for 10 minutes at 50°C. The solution is placed in the apparatus to be injected in the laminar flow vector fluid in a capillary. Somatic cells separated by the flow are exposed to a laser beam via a microscopic lens. The luminous impulses, emitted by the fluorescing somatic cells, are filtered and amplified by a photomultiplier, counted and converted to ICC / ml. A calibration enables the transformation from ICC / ml to cells / ml.

The apparatus is connected to a computer that ensures the running of the instrument and the signal treatment.

1/- THE TESTS

The evaluation tests were performed in CECALAIT's physico-chemical and microbiological laboratories in April 2008 and concerned the evaluation of the linearity, repeatability and accuracy.

ISO 13366 / IDF 148 and IDF 128 standards were taken as references.

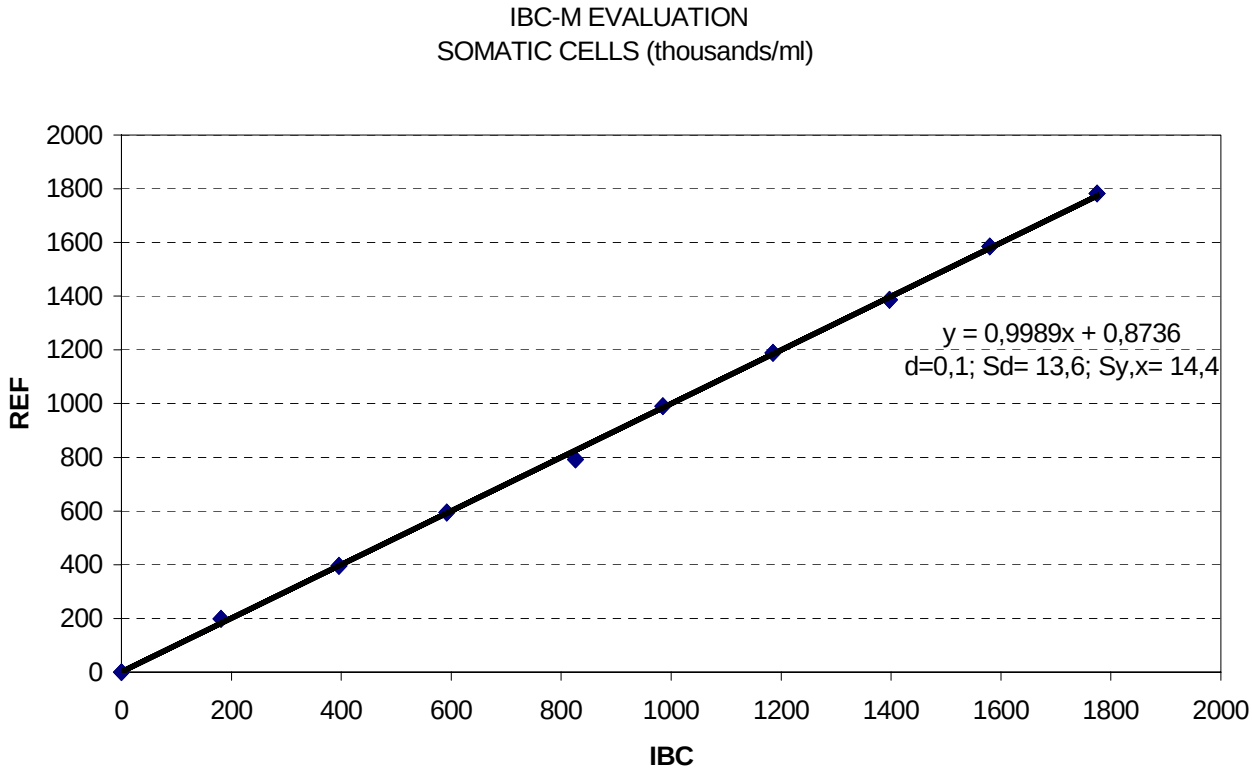
2/- THE SAMPLES

About 120 samples of individual cow milk from 2 farms in the Jura were collected during milking and preserved with Bronopol (0.02 % final). The samples were divided into several sets and stored at 4°C before analysis.

3/- CALIBRATION OF THE INSTRUMENT

IBC-M was first calibrated by the manufacturer between 0 and 1800 000 cells /ml with 10 commercial samples produced by CECALAIT.

Graph 1 presents the results obtained:



Graph 1: Calibration of IBC-M with CECALAIT's samples
d and Sd: mean and standard deviation of deviations (apparatus – reference); Sy,x: residual standard deviation of the linear regression REF vs IBC-M

It can be noted that the apparatus is linear in this measuring range. The mean of the deviations (d) is equal to 100 cells / ml (< 0.1 %), the residual standard deviation (Sy,x) is equal to 14 000 / ml, the slope is equal to 0.999 and the intercept is equal to 1 000 / ml. These parameters are in accordance with the CNIEL's requirements that fix the limits of 5 %, 15 000 / ml and 1000 +/- 5 %, respectively.

4/- REPEATABILITY

The repeatability was evaluated by the analysis, in consecutive duplicate, of a set of samples prepared in paragraph 2.

The results are presented in the table below:

Range (10^3/ml)	N	M (10^3/ml)	Sr (10^3/ml)	Sr (%)	r (10^3/ml)
0-2000	**123**	**111**	**1.9**	**1.7**	**5.3**
1-2000	96	142	2.1	1.5	5.8
1-100	56	37	1.7	4.7	4.7
1-300	86	81.6	1.8	2.2	5.0
1-400	89	91	1.9	2.0	5.3
1-500	91	99	1.9	2.0	5.3
401-2000	7	789	4.3	0.5	11.9

Table 1: Repeatability criteria of IBC-M

Range (10^3/ml)	N	M (10^3/ml)	Sr (10^3/ml)	Sr (%)	r (10^3/ml)
1-100	56	37	1.7	4.7	4.7
100-200	21	139	1.8	1.3	5.1
200-400	12	262	2.4	0.9	6.6
400-750	4	508	3.3	0.7	9.2
750-1500	2	767	4.2	0.5	11.6
1500-2000	1	1958	6.9	0.4	19.2

Table 2: IBC-M repeatability criteria (Control milk classes)

N: number of results; M: mean values; Sr (Sr%): absolute standard deviation of repeatability (relative); r: maximum deviation of repeatability in 95% of cases

In the range 0 to 2 000 000 cells / ml (mean 111 000 / ml), the repeatability of the IBC-M apparatus (1.7%) is up to IDF 148 standard specifications that fix the relative standard deviation of repeatability at 5%. It also satisfies the manufacturer's specifications concerning the SCC 150, fixed at 5% (< 100 000 / ml), at 3% (< 300 000 / ml) and 2% (< 500 000 / ml). These results are also in accordance with the CNIEL's specifications fixing the limit concerning the standard deviation of repeatability at 5%.

5/- ACCURACY

About 100 individual cow milk samples were selected among 120 samples used in paragraph 2 and were analysed, singly, by microscopic enumeration in accordance with the IDF 148 A method. In case of a more important residue after regression, a second count was realised.

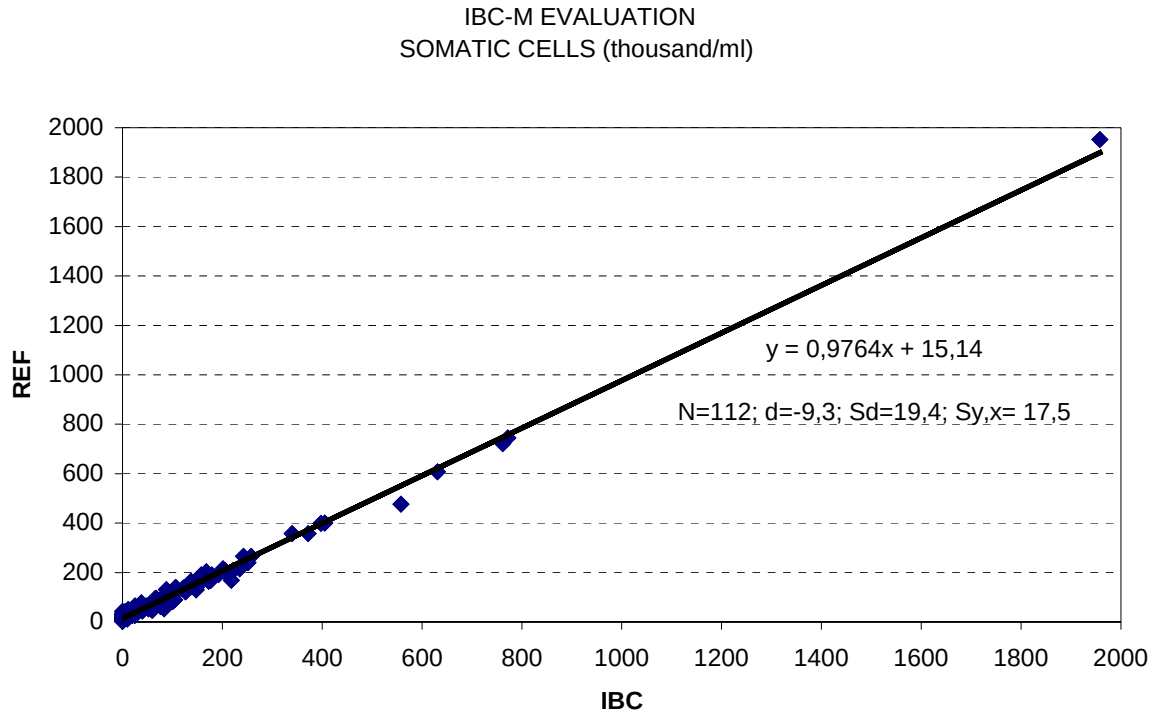
Table 3 summarizes the results obtained:

Measurement range (10^3/ml)	N	M (10^3/ml)	d (10^3/ml)	Sd (10^3/ml)	Sy,x (10^3/ml)
0-2000	112	127	-9.3	19.4	17.5
0-100	72	42	-13.8	14.4	12.0
0-400	107	91	-11.4	16.2	14.9
401-2000	5	901	35.4	28.1	24.3
100-2000	40	281	-1.1	24.1	22.6

Table 3: IBC-M accuracy criteria

N: number of results; m: mean of the reference values, d and Sd: mean and standard deviation of deviations (apparatus – reference); Sy,x: residual standard deviation of the linear regression REF vs IBC-M

Graph 2 below presents the relation between IBC-M and the microscopic enumeration and gives the parameters of the linear regression REF vs IBC-M.



Graph 2: Relation between the IBC-M somatic cells results and microscopic enumeration (ISO 13366/IDF 148)
N: number of results; d and Sd: mean and standard deviation of deviations (apparatus – reference); Sy,x: residual standard deviation of the linear regression REF vs IBC-M

Globally, for a range of 0-2000 thousand / ml, it can be noted that the mean deviation (d) is close to –10 000 / ml and the standard deviation of deviations (Sd) is about 19 000 / ml. The linear regression slope, equal to 0.976, is close to 1 and the intercept point is equal to 15 000 / ml.

GENERAL CONCLUSION

BactoCount IBC-M, evaluated on the request of BENTLEY for the enumeration of somatic cells, globally satisfies the linearity, repeatability and accuracy specifications announced by the manufacturer as well as the specifications with regards to repeatability and calibration used within the framework of instrument evaluation for authorisation of use for the purposes of milk payment in France and for milk control.

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