# CECALAIT-S NEWSLETTER

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CENTRE D'EXPERTISE ET DE CONTROLE DES ANALYSES LAITIERES

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**ARTICLE**

# EVALUATION OF BACTOCOUNT IBC-M

# FOR THE ENUMERATION OF TOTAL FLORA

**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.**
**The sample is collected and manually blended with an incubation reagent made up of a clarification buffer, a proteolytic enzyme and a fluorescent stainer. This reagent lyses somatic cells, scatters the proteins and fat, makes the bacteria wall permeable and colours their DNA.**
**The mixture is then incubated for 10 minutes at 50°C and manually blended twice with an ultrasonic probe. The solution is then placed in the apparatus to be injected in a laminar flow vector fluid in a capillary. Bacteria separated by the flow are exposed to the laser beam via a microscopic lens. The luminous impulses emitted by the fluorescing bacteria are filtered and amplified by a photomultiplier, counted and converted to Individual Bacteria Cells (IBC) per ml. A calibration performed by the laboratory permits a transformation from IBC/ ml to CFU / ml.**

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

All the tests constituting the evaluation were performed with samples without prior reheating.

Repeatability and accuracy were tested using as references ISO 4833, ISO 21187/IDF 196 and IDF 128 standards.

## 1) REPEATABILITY

### 1.1) Procedure

The repeatability was evaluated by the analysis, in consecutive duplicates, of 106 samples of herd milk in March 2008 and 50 samples from CECALAIT's proficiency tests on micro-organisms at 30°C from June 2007 to March 2008

### 1.2) Results

The table below presents the standard deviation of repeatability Sr (log CFU / ml) and the maximum deviation between duplicates, expressed by r (log CFU / ml) and by RD95 (% CFU / ml), for each concentration level. These levels were established according to their correspondences to milk payment classes in France.
It must be noted that 2 outliers were eliminated by the Cochran test at 1%.

| CFU classes / ml according to the reference method (Log) | n | Mean (Log) | Sr (Log) | r (Log) | RD 95 (%) |
|---|---|---|---|---|---|
| **All levels** | **154** | **4.384** | **0.072** | **0.198** | **58** |
| $0 - 50\ 10^3$ (0 – 4.699) | 108 | 4.053 | 0.082 | 0.229 | 69 |
| $50\ 10^3 - 100.10^3$ (4.699 – 5.000) | 20 | 4.843 | 0.035 | 0.098 | 25 |
| $100.10^3 - 300.10^3$ (5.000 – 5.477) | 20 | 5.244 | 0.035 | 0.097 | 25 |
| $> 300.10^3$ ( > 5.477) | 6 | 5.933 | 0.032 | 0.089 | 23 |

*n: Number of samples; Sr: Standard deviation of repeatability in log; r: Repeatability in log; RD 95: Maximum deviation between duplicates in 95 % of cases in % CFU / ml*

### 1.3) Conclusion

Over all the ranges tested (mean contamination rate of 24 000 CFU/ml), the instrument presents a standard deviation of repeatability Sr of about 0.072 Log that corresponds to the technical specifications announced by the manufacturer (Sr ≤ 0.07 Log) and is lower than the limit generally required within the context of milk payment in France for this determination (Sr ≤ 0.12 log).
For contamination rates above 50 000 CFU / ml, the standard deviation of repeatability is below 0.035 log.

## 2) ACCURACY

The accuracy was estimated from the residual standard deviation of regression using the reference method (Log CFU/ ml) as the expected value Y and BactoCount IBC-M as the explanatory variable X (Log CFU/ ml).

### 2.1) Herd milk samples

#### 2.1.1) Procedure

140 samples of cow herd milk were analysed on 3 different days in March 2008. Each analytical series was constituted of milk from a collecting round (storage 24 or 48 hours in tank) sampled in duplicate on the cattle-farms and following the normal sample network for milk payment.

For a population presenting a good distribution in levels of total flora, 108 samples out of 140 were selected on the basis of the results obtained using the BactoCount IBC-M instrument.

The analyses were performed in consecutive duplicate using the instrument and the reference method (ISO 4833).

For the evaluation of accuracy, only the samples with a reference value technically validated were conserved after elimination of dishes outside the counting limits or presenting intrusive colonies.
As one sample using the reference method (< 4 colonies/ dish) and one sample using the instrumental method (IBC result) were not taken into account, 106 samples / 108 selected were retained for the accuracy study.

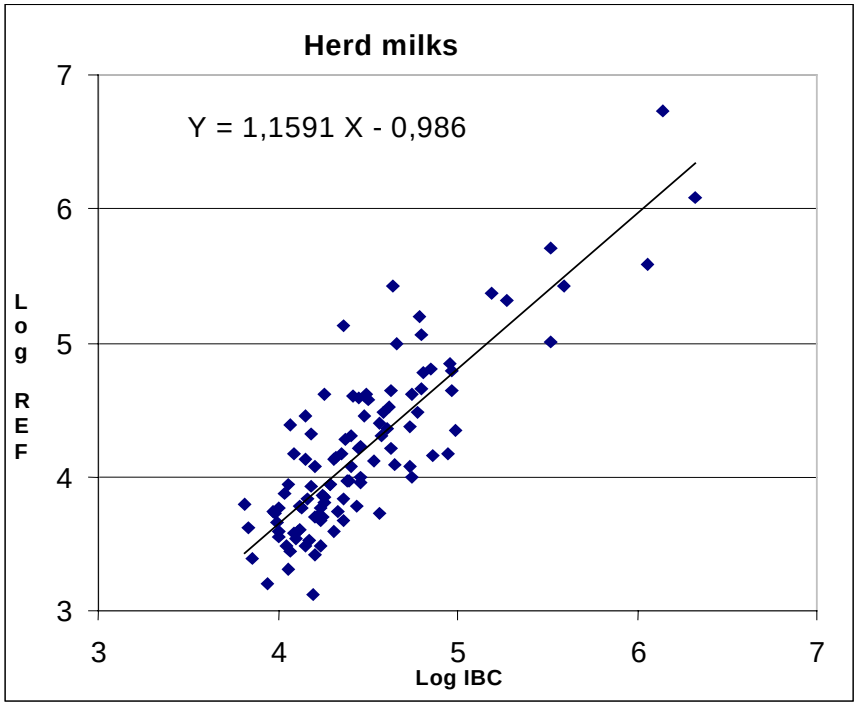
#### 2.1.2) Results

4 outliers were removed from the treatment:
- 2 samples eliminated by the Cochran test at 1%
- 2 samples presenting a standard deviation between the methods higher than 3 times the residual standard deviation ($S_{y,x}$)

The simple linear regression calculated for the population of the remaining 102 herd milk samples gave the following relation:
Log (Reference) = **1.1591** x Log (IBC-M) **- 0.986**
**$S_{y,x}$ = 0.311 Log**
Estimation precision: ± 1.96 x 0.311
that is **± 0.61 Log CFU /ml**

*Characteristics of the 102 samples:*

*Mean value: 15 .10 $^3$ CFU / ml (Log: 4.176)*
*Minimum value: 1.3.10 $^3$ CFU / ml (Log: 3.128)*
*Maximum value: 5 450.10 $^3$ CFU / ml (Log: 6.736)*



**2.2) Samples from CECALAIT's proficiency tests**

2.2.1) Procedure

The analyses were performed on 50 samples from CECALAIT's proficiency tests from June 2007 to March 2008, representing 5 proficiency tests of 10 samples. The analyses were realised in consecutive duplicate on the instrument and singly with the reference method (ISO 4833).

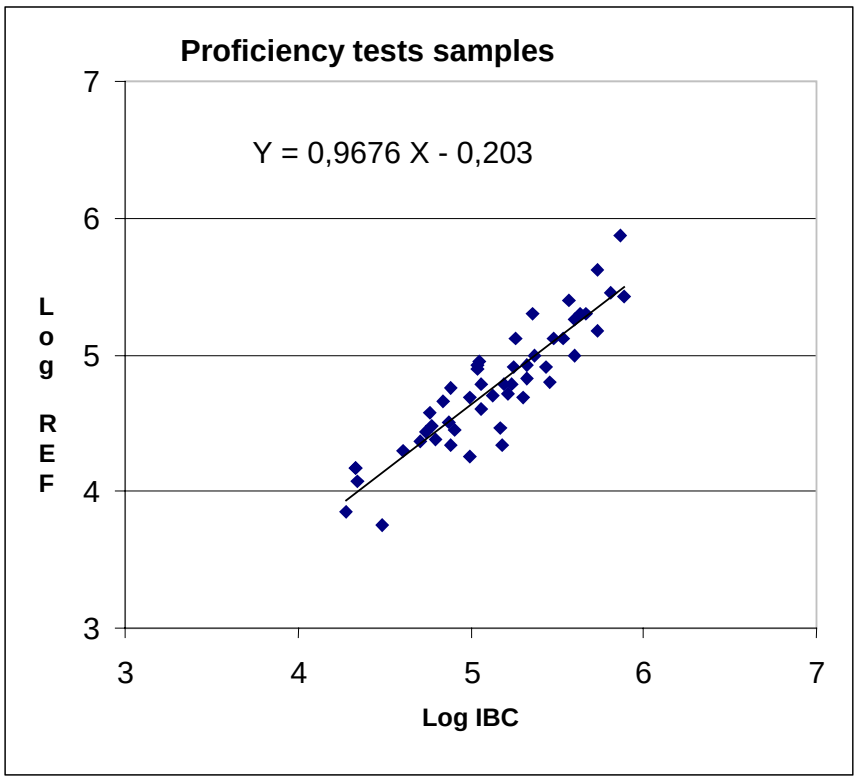
2.2.2) Results

Any outliers were removed from the treatment.

The simple linear regression calculated for the population of 50 samples gave the following relation:
Log (Reference) = **0.9676** Log (IBC-M) **– 0.203**
**$S_{y,x}$ = 0.194 Log**
Estimation precision: ± 1.96 x 0.194
that is **± 0.38 Log CFU /ml**

*Characteristics of the 50 samples:*

*Mean value: 59 .10 $^3$ CFU / ml (Log: 5.875)*
*Minimum value: 6 .10 $^3$ CFU / ml (Log: 3.756)*
*Maximum value: 750 .10 $^3$ CFU / ml (Log: 6.736)*




**2.3) Herd milk and proficiency test samples**

2.3.1) Procedure

The treatment of accuracy of the 106 samples of cow herd milk and the 50 samples of CECALAIT's proficiency tests was carried out according to the procedure described above.

2.3.2) Results

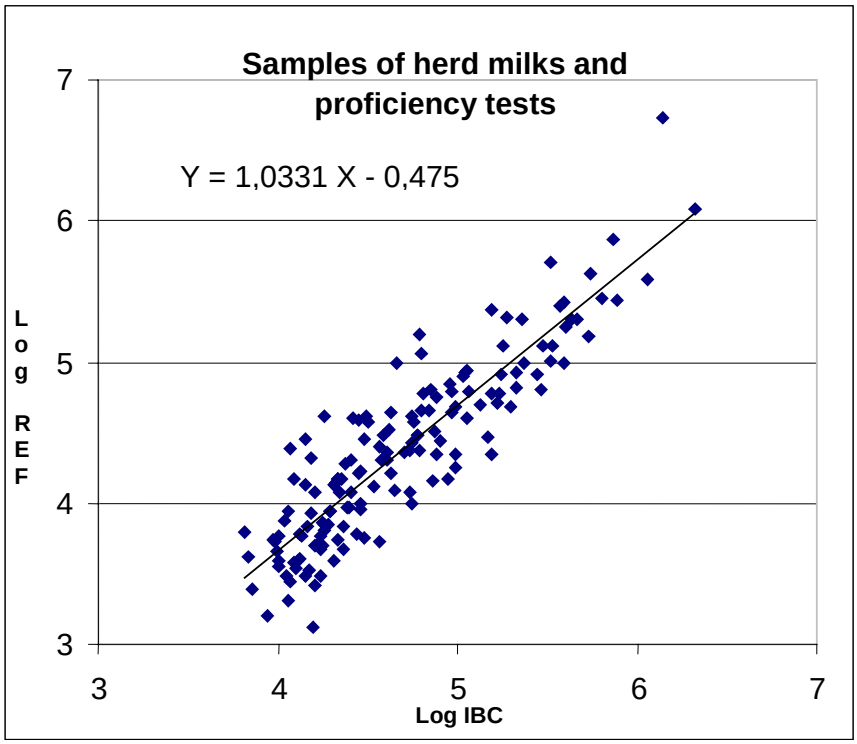
4 outliers were removed from the treatment:
- 2 samples eliminated by the Cochran test at 1%
- 2 samples presenting a deviation between the methods higher than 3 times the residual standard deviation ($S_{y,x}$).

The simple linear regression calculated for the population of the 152 remaining samples gave the following relation:

Log (Reference) = **1.0331** x Log (IBC-M) **- 0.475**
**$S_{y,x}$ = 0.285 Log**
Estimation precision: ± 1.96 x 0.285
that is **± 0.56 Log CFU /ml**

*Characteristics of the 152 samples:*

*Mean value: 24 .10 $^3$ CFU / ml (Log: 4,372)*
*Minimum value: 1.3.10 $^3$ CFU / ml (Log: 3,128)*
*Maximum value: 5 450.10 $^3$ CFU / ml (Log: 6,736)*




### 2.4) Conclusion

For the 152 samples, the instrument presents a residual standard deviation $S_{y,x}$ of 0.285 Log (for a mean contamination of 24 000 CFU / ml), in compliance with the technical specifications of the manufacturer (Sy,x ≤ 0.30 Log) and the limit fixed within the framework of the evaluation of instruments for the authorisation of use for the purposes of milk payment in France (Sy,x ≤ 0.30 Log).

It can be noted that the residual standard deviation obtained for the 50 proficiency test samples is weaker than the value obtained for the 102 herd milk samples (0.194 Log against 0.311 Log). This is due to the presence of a much larger diversity in flora in the latter type of milk and a much lower mean contamination level ($15.10^3$ against $59.10^3$)

## GENERAL CONCLUSION

BactoCount IBC-M instrument evaluated on the request of BENTLEY for the enumeration of total flora globally satisfies the repeatability and accuracy specifications announced by the manufacturer and those used within the framework of the evaluation of instruments for authorisation of use for the purposes of milk payment in France.

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# 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.

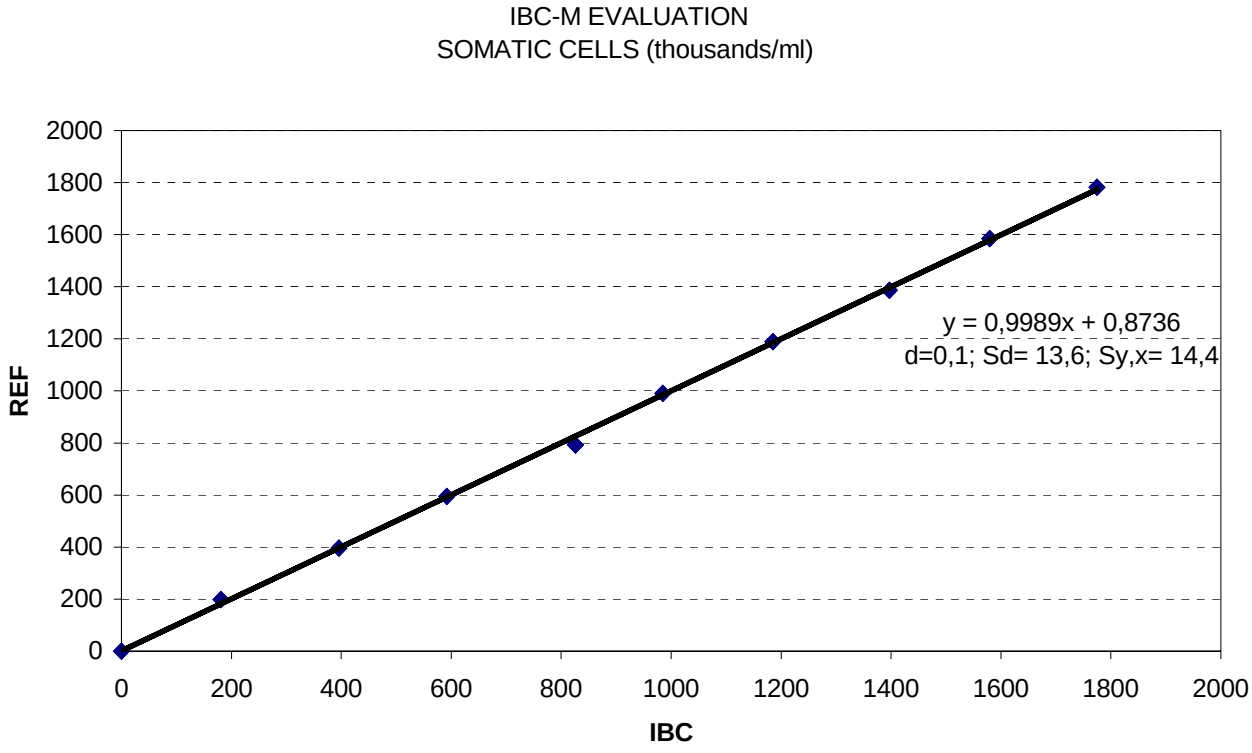
## 2/- THE SAMPLES

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.

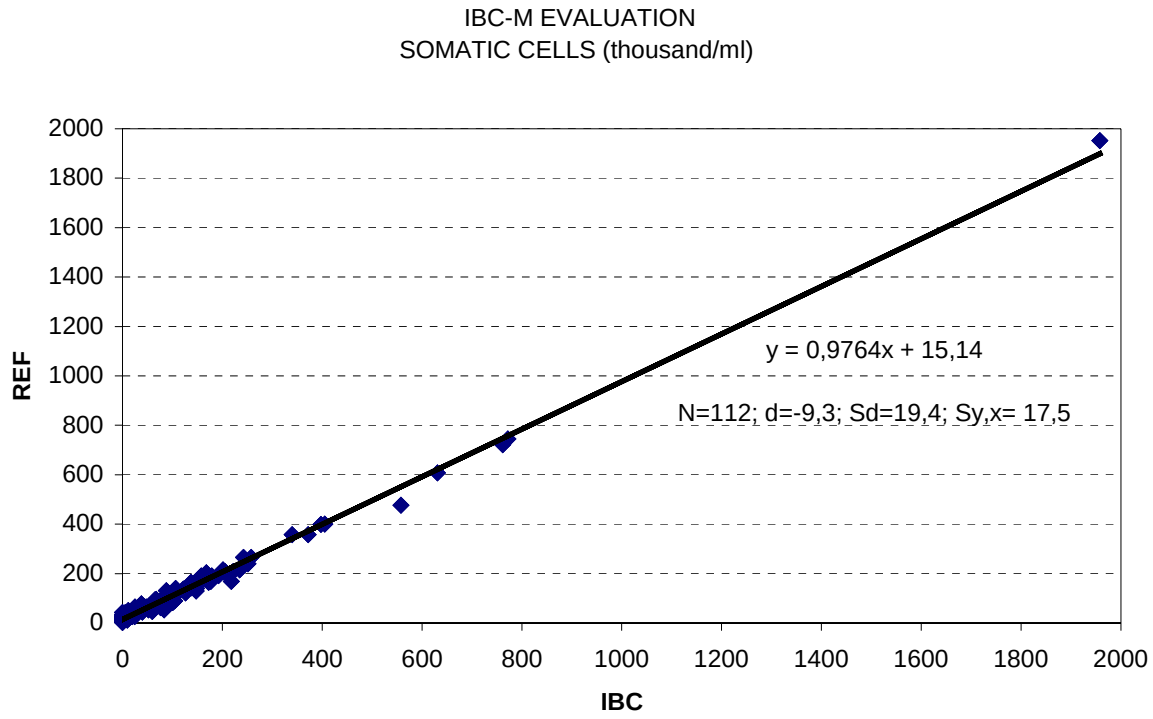
Table 3 summarizes the results obtained:

| 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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# STANDARDS, DRAFT STANDARDS

**Classification in alphabetic order by theme**

## ISO published standards

| MILK AND MILK PRODUCTS | | |
|---|---|---|
| MILK / MILK PRODUCTS / NITROGEN / PROTEINS | ISO/TS 17837:2008 March 2008 | MILK AND MILK PRODUCTS Determination of nitrogen content and crude protein calculation – Kjeldahl method |

# NEW EU REGULATIONS

**Classification is established in alphabetical order of the first keyword**

**ANALYSIS METHODS** / MILK / DAIRY PRODUCTS

**O.J.E.U. L 88, 29th March 2008** – Commission Regulation (EC) n° 273/2008 of 5 March 2008 laying down detailed rules for the application of Council Regulation (EC) n° 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:088:0001:0115:EN:PDF

**PROCESSING** / MILK / DAIRY PRODUCTS

**O.J.E.U. L 132, 22nd May 2008** – Commission Regulation (EC) n° 437/2008 of 21 May 2008 amending annexes VII, X and XI to Regulation (EC) n° 1774/2002 of the European Parliament and of the Council as regards the requirements for the processing of milk and milk products defined as Categorie 3 material
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:132:0007:0013:EN:PDF

**PROTECTION OF GEOGRAPHICAL INDICATIONS**

**O.J.E.U. C 85, 4th April 2008** – Publication of an application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [San Simon da Costa (cheese) (PDO)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2008:085:0013:0016:EN:PDF

**O.J.E.U. C 111, 6th May 2008** – Publication of an amendment application pursuant to Article 6 (2) of Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs [Gorgonzola (cheese) (PDO)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2008:085:0013:0016:EN:PDF

**O.J.E.U. L 125, 9th May 2008** – Commission Regulation (EC) n° 417/2008 of 8 May 2008 amending Annexes I and II to Council Regulation (EC) n° 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:125:0027:0027:EN:PDF

**O.J.E.U. L 143, 3rd June 2008** – Commission Regulation (EC) n° 487/2008 of 2 June 2008 registering a name in the register of protected designations of origin and protected geographical indications [Casatella Trevigiana (cheese) (PDO)]
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:143:0012:0012:EN:PDF

**SWEETENERS**

**O.J.E.U. L 158, 18th June 2008** – Commission Directive 2008/60/EC of 17 June 2008 laying down specific purity criteria concerning sweeteners for use in foodstuffs
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:158:0017:0040:EN:PDF

**VETERINARY MEDICINAL PRODUCTS** / M.R.L.

**O.J.E.U. L 116, 30th April 2008** – Corrigendum to Commission Regulation (EC) n° 508/1999 of 4 March 1999 amending Annexes I to IV of Council Regulation (EEC) n° 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:116:0086:0086:EN:PDF

**O.J.E.U. L 157, 17th June 2008** – Commission Regulation (EC) n° 542/2008 of 16 June 2008 amending Annexes I and II to Council Regulation (EEC) n° 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, as regards cyfluthrin and lectin extracted from red kidney beans (*Phaseolus vulgaris*)
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:157:0043:0045:EN:PDF

# BOOKSHOP: LATEST PUBLICATIONS

The classification in alphabetic order of the first keyword allows you to consult the references according to your interests. The web site allows you to know more, or to order the book.

## LIQUID CHROMATOGRAPHY

COHEN S.E.; SCHURE M.R. – **Multidimensional liquid chromatography: theory and applications in industrial chemistry and the life sciences** –Wiley Editions – April 2008 – ISBN 978-0-471-73847-3 – 456 pages

http://eu.wiley.com

This book presents instrumentations, theory, methods development, and applications of multidimensional liquid chromatography in various fields.

## *LISTERIA MONOCYTOGENES*

LIU D. – **Handbook of *Listeria monocytogenes*** –CRCPress Editions – April 2008 – ISBN 9781420051407 – 552 pages

http://www.crcpress.com

This book divided into 4 sections discusses the pathogenicity of this bacterium, demonstrates identification and detection techniques, details the current knowledge and examines the different possibilities of use of this bacterium

# FORTHCOMING EVENTS

Classified in chronological order

## DAIRY PRODUCTS

| | | |
|---|---|---|
| 9-14 November 2008<br>Mexico City, Mexico | IDF World Dairy Summit | http://www.wds2008mexico.com |

## DAIRY PRODUCTS / MINERALS

| | | |
|---|---|---|
| 1-3 October 2008<br>Saint-Malo, France | 1st International Symposium<br>on minerals ans dairy products | http://www.inra.fr/mdp2008 |

# IN THE PRESS – ON THE WEB

**Classification in alphabetical order of keywords**

## STANDARD / CODEX

### Report of the eighth session of the Codex Committee on milk and milk products

**http://www.codexalimentarius.net/web/archives.jsp?year=08**

► This session was held from 4 to 8 February 2008 at Queenstown in Nouvelle-Zélande. The matters for adoption by the 31th session of the Codex Alimentarius Commission are described in the part A. Part B concerns requests for comments and information from governments and interested international organisations.

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