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.

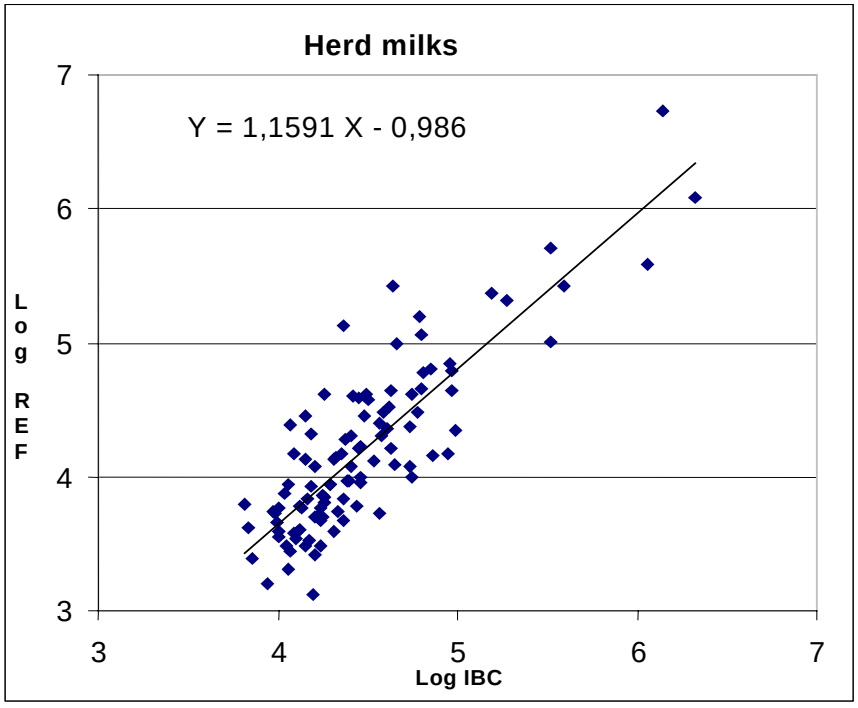
2.1.2) Results

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).

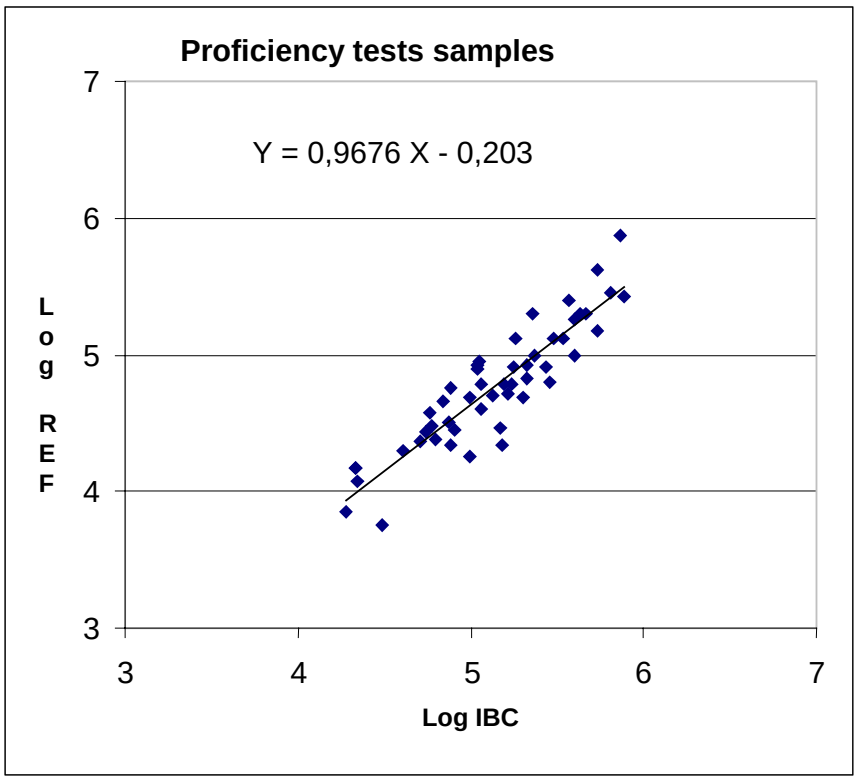
2.2.2) Results

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

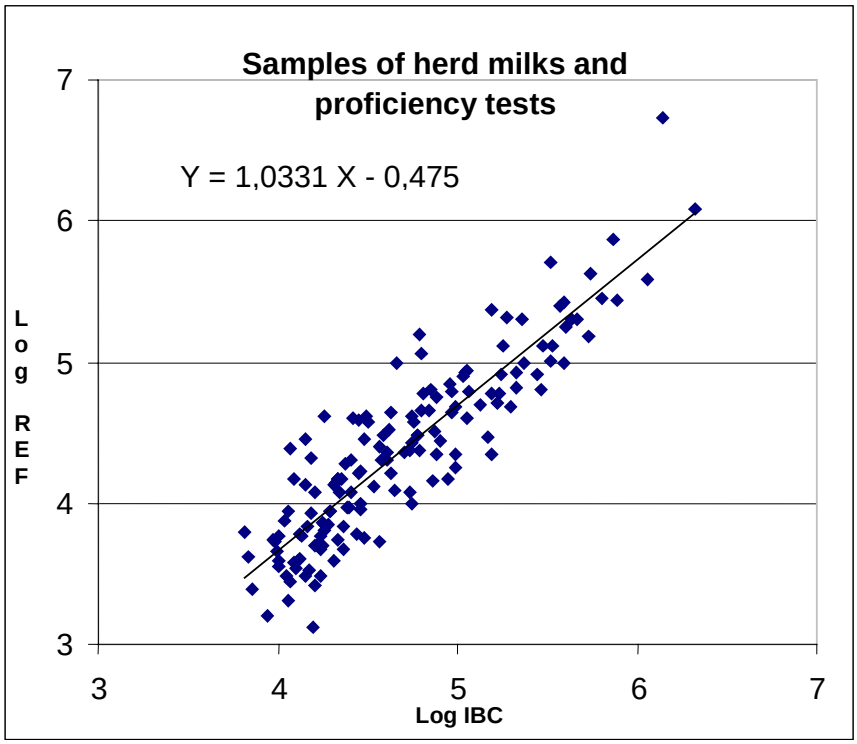
4 outliers were removed from the treatment:
- 2 samples eliminated by the Cochran test at 1%
- 2 samples presenting a deviation between the methods higher than 3 times the residual standard deviation ($S_{y,x}$).

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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