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MICRO ORGANISM
Total flora

REFERENCE METHOD(s)
EN-ISO 4833-1:2013
EN-ISO 4833-2:2013

SCOPE
Raw cow milk

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Summary of the validation of the BactoCount IBC and IBCm (Bentley Instruments) against the EURL MMP criteria for determination of total flora in raw cow milk

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1. Principle of the alternative method

The BactoCount is a (fully) automated flow cytometer for the rapid enumeration of individual bacteria in raw milk. The raw milk is sampled and dispensed into individual wells located on a carousel with temperature regulated at 50°C. There the raw milk sample is mixed with an incubation reagent. The incubation reagent contains a clarification buffer, a proteolytic enzyme, and a fluorescent marker. The reagent serves to lyse the somatic cells, to solubilize the fat globules and proteins, to permeabilize the bacterial cell walls and to stain their DNA. The fluorescence marker intercalates rapidly and selectively with the bacterial DNA. The mixture is then sonicated twice during the incubation. The sonication process promotes the chemical breakdown of the interfering particles and disrupts the remaining bacteria cells to improve the detection of individual bacteria and reduce the background fluorescence. The cell debris, devoid of nucleic acid, becomes excluded from the analysis.

After the incubation, the mixture is transferred automatically to the flow cytometer where the bacteria are aligned and exposed to an intense laser beam which causes them to fluoresce. The fluorescence signal is collected by the optics, filtered, and detected with a photo multiplier. The fluorescence pulses intensity and height are recorded and used as gating parameters. The sorted pulses are then translated into individual bacteria count (IBC) and converted to CFU (reference scale) after applying a conversion equation. A “universal” conversion equation developed on a large database of samples representative of all potential sources of variation in the milk flora (according to ISO 21187|IDF 196) is installed on the instrument as a startup conversion equation (6).

BactoCount is represented with two models: BactoCount IBC is fully automated and BactoCount IBCm is semi-automated (sample preparation is manual). The two models have no differences in their applications and technical principal. Both models of BactoCount instruments are operating with the same conversion equation.

This document is a summary of the method comparison study and the interlaboratory study of the BactoCount IBC/IBCm (Bentley Instruments) against the criteria in the EURL MMP document from December 2011. The MicroVal validation report presents the full results of the validation.

2. Scope

Raw cow milk

3. Result and conclusions

3.1. Method comparison study

BactoCount IBC/IBCm performance characteristics determined according to ISO 16297 are:

- Lower limit of quantification: 18.10^3 IBC/ml (5.10^3 cfu/ml)
- Detection limit: 7.10^3 IBC/ml (2.10^3 cfu/ml)
- Upper limit of quantification: $1{,}2.10^7$ IBC/ml (3.10^6 cfu/ml)

- Linearity in the working range: 2,11 %
- Carry-over effect, COR: 0,18 % (EURL MMP criterion: COR < 1 %)
- Repeatability (*r*): 0,08 – 0,20 $\log_{10}$ cfu/ml
  (EURL MMP criterion: *r* =0,25 $\log_{10}$ cfu/ml)

With regard to the agreement between the reference method (ISO 4833-1:2013) and the alternative method:

- the alternative method fulfils the accuracy criterion of ISO 16297 and the EURL MMP document in the range of interest (1.10^4 cfu/ml - 1.10^6 cfu/ml). The BactoCount IBC/IBCm accuracy standard deviation is $s_{y,x}$<0,34 $\log_{10}$ cfu/ml, better than the limit $s_{y,x}$<0,40 $\log_{10}$ cfu/ml. required by the EURL MMP document.
- with applying an adequate conversion, the alternative method is not biased with respect to the reference method.

3.2. Interlaboratory study

The Interlaboratory Study was performed according to ISO/DIS 16140-2:2013. The conclusions are:

- The repeatability standard deviation with the alternative method is better than with the reference method. The average repeatability (*r*) across levels is 0,10 $\log_{10}$ cfu/ml for the alternative method and 0,18 for the reference method (required *r*=0,25 $\log_{10}$ cfu/ml according to ISO 4833-1:2013 and ISO 16140-2:2013).
- The reproducibility standard deviation with the alternative method is better than with the reference method. The average reproducibility (*R*) across levels is 0,12 $\log_{10}$ cfu/ml for the alternative method and 0,25 $\log_{10}$ cfu/ml for the reference method (required *R*=0,45 $\log_{10}$ cfu/ml according to ISO 4833-1:2013 and ISO 16140-2:2013).
- With applying an adequate conversion, the alternative method is not biased with respect to the reference method with a maximum bias of 0,13 $\log_{10}$ cfu/ml, better than the limit <0,5 $\log_{10}$ cfu/ml required by ISO 16140-2:2013.

4. Final conclusion of the validation study

The Method Comparison Study and the Interlaboratory Study show that the alternative method results obtained with BactoCount IBC/IBCm (Bentley Instruments) comply with the criteria of the EURL MMP document.