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MICRO ORGANISM
Total flora

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EN-ISO 4833-2:2013

SCOPE
Raw cow milk

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MicroVal validation of the BactoScan FC and FC+ (FOSS Analytical A/S) against the EURL MMP criteria for determination of total flora in raw cow milk

Summary of the MicroVal validation of the BactoScan FC and FC+ (FOSS Analytical A/S) against the EURL MMP criteria for determination of total flora in raw cow milk

1. Principle of the alternative method

The BactoScan is a fully automated flow cytometer for the rapid enumeration of individual bacteria in raw milk. The raw milk is sampled and pretreated in an incubation section to break down possibly interfering components. This is achieved by a combined chemical and enzymatic treatment. Additionally, the mixture undergoes a mechanical treatment to disrupt the bacteria cells. The bacteria are subsequently stained with the fluorescent dye ethidium bromide. All the reagents involved are automatically filtered directly prior to their application to eliminate the risk of contamination from other sources than the milk.

A precise syringe is used to pass the sample through a flow cell, presenting the bacteria one by one to blue light from a laser source. The stained bacteria are excited by the blue light and emit red light with one light pulse for every bacteria passing the laser beam.

The fluorescent light is detected by a highly sensitive detector (Photo Multiplier Tube - PMT), which generates electronic impulses. The electronics count the pulses and display them in a pulse height analysis (PHA) diagram.

With the BactoScan FC/FC+ an Individual Bacteria Count is determined, whereby results are displayed in IBC/ml. In order to convert IBC/ml to cfu/ml a conversion equation is applied. Guidelines for the establishment and maintenance of a conversion equation are described in ISO 21187|IDF 196. BactoScan is represented with two fully automated models: BactoScan FC and BactoScan FC+. The two models have no differences in the technical principal and in their application.

This document is a summary of the results of a method comparison study and an interlaboratory study with the BactoScan FC/FC+ (FOSS Analytical A/S) 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

BactoScan FC/FC+ performance characteristics determined according to ISO 16297|IDF 161 are:

- Lower limit of quantification: $2{,}8.10^4$ IBC/ml ($9{,}6.10^3$ cfu/ml)
- Limit of detection: 10.10^3 IBC/ml ($3{,}8.10^3$ cfu/ml)
- Upper limit of quantification: $1{,}9.10^8$ IBC/ml ($2{,}9.10^7$ cfu/ml)
- Linearity in the working range: 2,36 %
- Carry-over effect, COR: 0,31 % (EURL MMP criterion: *COR* < 1%)
- Repeatability (*r*): 0,03 – 0,15 $\log_{10}$ cfu/ml (EURL MMP criterion: $r < 0{,}25$ $\log_{10}$ cfu/ml)

Remark: The lower limit of quantification and the limit of detection are calculated with the results obtained from a single raw cow milk sample with an average bacterial count of 5300 IBC/ml (2.10^3 cfu/ml).

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

- the alternative method fulfils the accuracy criterion of ISO 16297|IDF 161 and the EURL MMP document in the range of interest (1.10^4 cfu/ml - 1.10^6 cfu/ml). The BactoScan FC/FC+ accuracy standard deviation is $s_{y,x}$<0,28 $\log_{10}$ cfu/ml, better than the limit $s_{y,x}$<0,40 $\log_{10}$ cfu/ml required by the EURL MMP document.
- when 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 0,07 $\log_{10}$ cfu/ml for the alternative method and 0,18 $\log_{10}$ cfu/ml 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 0,08 $\log_{10}$ cfu/ml for the alternative method and 0,52 $\log_{10}$ 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).

  The rather large average reproducibility (R) of the reference method underlines the good performance of the BactoScan FC/FC+ and its independence from external factors (e.g. technicians, instruments etc.).
- With applying an adequate conversion, the alternative method is not biased with respect to the reference method (max bias = -0,15 $\log_{10}$ cfu/ml, acceptability limit is set at: AL = ±0,5 $\log_{10}$ units according to ISO 16140-2:2013).

4. Final conclusion validation study

The Method Comparison Study and the Interlaboratory Study show that the alternative method results obtained with BactoScan FC/FC+ (FOSS Analytical A/S) comply with the criteria of the EURL MMP document.