



Technical Sheet

Milk Standards (“Bacteria controlmilk”)

Control solution for flow cytometry instruments, like BSC-FC, BC,..

Composition

Raw milk with definite cultures of bacteria; preserved with *Acidiol* (“Na-Azid”) *)

- not suitable for consumption

Samples

Two (three) samples with different IBC – levels

- ❖ Milkstandard A : BSC FC: 140 – 190 IBC/µl (BC: 170-235 IBC/µl)
- ❖ Milkstandard B : BSC FC: 400 – 500 IBC/µl (BC: 490-610 IBC/µl)
- ❖ Milkstandard C**) : BSC FC: 800-1.200 IBC/µl (BC: 975 – 1.465 IBC/µl)

Application - delivery

every 4 weeks – 24 countries, 70 laboratories, 120 instruments

- ❖ Control milk for the daily measuring routine
- ❖ interlaboratory studies – detailed statistics every 4 weeks.

*) Dosage: 0,3% (0,00005% of Na-Azid)

**) special sample for checking the carry-over of samples with high germ contents

***) not part of the accreditation

BSC FC: BactoScan, **BC:** BactoCount

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Accuracy - precision

- ❖ VK% r : Standard deviation of repeatability/arithm. mean * 100 : 2,5-4,5 %
- ❖ VK% R: Standard deviation of reproducibility/arithm. mean * 100 : 4,5-5,5%
- ❖ Uncertainty of measurement (reproducibility log sR x 2) : 0,04-0,06 log
~ +/- 10% (VK% R x 2)


Correctness – Calculation of a standard value

The nominal value is determined on the basis of the following methods:

- a) reference method (Plate count method). Because of the known germ flora (special lactic acid bacteria) of the Milkstandard the determination of the viable count is possible within an accuracy of $\pm 5\%$. *The Microbiology – the Total Count methods are accredited according DIN EN ISO 17025.*
- b) Further: by a fluoreszensoptic method: BacSomatic instrument (Foss,DK), in consideration of Milkstandard samples with defined, known measurement values.
- c) The reference value has a precision of $\pm 10\%$ (for each sample the uncertainty will be calculated).

Stability

In the refrigerator – at 6-8°C – you shouldn't store the Milkstandards for a longer time than 7 days (incl. delivery time). Possible are storing temperatures fo 2-4°C. However – we advise a frozen storing of the Milkstandards – at -18°C to – 30°C (after filling up in little portions). The filled portions will have a good stability for 6 months by about 3% decrease of the measuring values.

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Portioning – Fillig up in little units

The samples are sent in units of 900 ml.

The standard milk should be portioned in smaller units (of 40 ml each) and deep-frozen on the day of its arrival or the day after. The arrival time and temperature of the standards should be noted. The temperature should be lower than 10°C. Temperatures of more than 15°C are critical. If the Milkstandards are not in a satisfactory condition, we will send a replacement.


Due to the cold packs, ice can form locally. It is essential to ensure that the standards do not contain ice pieces when portioning and freezing, as this can cause the fat to crystallize (when thawing). The filling temperature must be between 5 ° C and 10 ° C.

The standard bottles have an empty head area. It is sufficient to turn around the 1-liter-bottles approx. 25 times prior to filling. Do not shake them! Too much stirring would rather be detrimental. The used bottles should be clean and dry and should not contain any residual detergents

Storage

It is recommendable to space the bottles in order to obtain a fast freezing. Temperatures from – 18°C to - 30°C are sufficient. **Please ensure of rapid freezing!** Before freezing, the milk in the small bottles should not be too warm. Temperatures of <6 ° C ensure a quick freezing process. The risk of creaming and segregation is lower. Sometimes the milk becomes gravelly or oily after thawing.

If the sample bottles (vials) have a fat film / fat collar, this fat layer can be dissolved again by briefly heating the sample to 36-40 ° C.

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Handling of the stored, frozen samples

It has proved to be effective to thaw out the standard samples overnight in the refrigerator (at 6-8°C). At his handling you should take care that the “Standard-Milks” are completely thawed! Alternatively it is possible to thawing up the Standard-milks in warm water (20-30°C for 10-20 minutes) – under occasionally swinging (carefully shaking). Bactocount samples you should put for a short (1-2 Minutes) into a waterbath (~40°C) – turning at least 10 times.

With the new generation of FC instruments it is possible, to measure the milksamples without a previous warming period. But the best results – regarding repeatability and reproducibility – you will get if you are measuring between at temperatures > 12°C. However, since the calibration milk samples have been deep-frozen before, a „warm-up period" of 10-20 minutes (optimal: 2-5 minutes at 40°C) will be of advantage. Longer standing times (> 60 minutes) at room temperature will result in higher measuring values!

If you are using a sonicator (by Bactocount) in front of measuring, you will usually get higher results due to the fact that most of the bacteria in raw milk are building chains and agglomerates (especially in cooled milk). The bacterias of the *Milk-Standards* have first of all the formation of “*Diplo-cocci*”, furthermore 10% short chains.

On their arrival, the standards can be examined for possible quality defects. In case of problems, you'll immediately receive a free-of-charge replacement. The examinations which are relevant for the comparative study should be carried out on already frozen standard milk samples

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Measurement of the Milkstandards and Controlmilk:

Approximately 5-10 measurements should be carried out from the "old" and "new" milk standard batches.

In addition to the „Milkstandard samples“ you will further receive control milk samples in special portions. These samples has to be stored and measured in the same way as the Milkstandards. If the measurement is made later, these samples should also be frozen.

It is an advantage if you use the .xls Formpapers (see: www.mih-huefner.de).

It is convenient if you also return a PHA image of the technical BZ standard and the respective milk standards.

Evaluations - results report

Every 4 weeks the laboratories get a detailed statistic resp. evaluation of the results you send in comparison with the results of other laboratories (in 24 countries). You get a special CODE for your instruments.

The results are evaluated monthly - with regard to: ***)

- a) Correctness of the measurement results (deviation – z-score - from total mean and setpoint)
- b) Repeatability (coefficient of variation for repeatability)
- c) Comparability (coefficient of variation for comparability)

***) not part of the accreditation

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Dr. Josef Hüfner

Reference material manufacturer accredited by DAkkS according to DIN EN ISO 17034



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