

JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials

CERTIFICATE OF ANALYSIS

ERM® - BD001a

MILK		
Cell concentration		
	Certified value ³⁾ [cells/mL]	Uncertainty ⁴⁾ [cells/mL]
Somatic cell count (SCC) ¹⁾	64000	8000
Somatic cell count (SCC) ²⁾	62000	6000
¹⁾ As defined in ISO 13366-1. The certified value is the mean value of 14 accepted data sets obtained from ISO 13366-1-compliant measurements. ²⁾ As defined in ISO 13366-1 and ISO 13366-2. The certified value is the mean value of 14 accepted data sets obtained from ISO 13366-1-compliant measurements and 14 data sets randomly selected out of 32 accepted data sets obtained from ISO 13366-2-compliant measurements. ³⁾ Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and with methods of determination referred to in footnotes 1 and 2. The certified value and its uncertainty are traceable to the International System of units (SI). ⁴⁾ The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.		

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 3 g.

Geel, January 2020

Signature **INFORMATION ONLY**

Dr Stefanie Trapmann
Head of Unit Reference Materials (acting)
European Commission, Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Retieseweg 111
B-2440 Geel, Belgium

DESCRIPTION OF THE MATERIAL

The CRMs are supplied in a set consisting of 1 bottle of ERM-BD001a and 1 bottle of ERM-BD001b. Each bottle contains 14 g of milk powder in an inert gas atmosphere.

ANALYTICAL METHODS USED FOR CERTIFICATION

The reference SCC method based on microscopy according to ISO 13366-1 and the routine SCC method based on fluoro-opto-electronic counting according to ISO 13366-2 were used. In some US laboratories, similar methods described in "Standard methods for the examination of dairy products" (chapters 10, direct microscopic SCC and 11.032, electronic SCC) were used.

PARTICIPANTS

Actalia Cecalait, Poligny, FR

(measurements under the scope of ISO/IEC 17025 accreditation by Cofrac; accreditation number 1-5577)

Agroscope, Bern, CH

(measurements under the scope of ISO/IEC 17025 accreditation by SAS; accreditation number STS 077)

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Bulgarian Food Safety Agency, Food Safety and Quality Laboratory, Sofia, BG

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Israeli Cattle Breeders' Association, Central milk lab, Caesarea, IL

(measurements under the scope of ISO/IEC 17025 accreditation by ISRAC; accreditation number 164)

Centre Wallon de Recherches Agronomiques, Gembloux, BE

(measurements under the scope of ISO/IEC 17025 accreditation by BELAC; accreditation number 189-TEST)

Delta Instruments B.V., Drachten, NL

Dairy laboratory Ltd., Ulbroka, LV

(measurements under the scope of ISO/IEC 17025 accreditation by LATAK; accreditation number LATAK-T-283-12-2003)

Eastern Laboratory Services (ELS), Medina, US

FOSS Analytical A/S, Hillerød, DK

INTI Lácteos, Rafaela, AR

(measurements under the scope of ISO/IEC 17025 accreditation by OAA; accreditation number LE 075)

Institute of Food Safety, Animal Health and Environment BIOR, Riga, LV

(measurements under the scope of ISO/IEC 17025 accreditation by LATAK; accreditation number LATAK-T-012-29-95)

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma, IT

(measurements under the scope of ISO/IEC 17025 accreditation by Accredia; accreditation number 0201)

Laboratoire interprofessionnel d'analyses laitières du massif central, Aurillac, FR

(measurements under the scope of ISO/IEC 17025 accreditation by Cofrac; accreditation number 1-0196)

Laboratorio agroalimentario de Santander, Santander, ES

(measurements under the scope of ISO/IEC 17025 accreditation by ENAC; accreditation number 517/LE1040)

Max Rubner-Institut, Bundesforschungsinstitut für Ernährung und Lebensmittel, Institut für Sicherheit und Qualität bei Milch und Fisch, Kiel, DE

(measurements under the scope of ISO/IEC 17025 accreditation by DAkkS; accreditation number D-PL-18156-02-00)

Melkcontrolecentrum Vlaanderen, Lier, BE

(measurements under the scope of ISO/IEC 17025 accreditation by BELAC; accreditation number 096-TEST)

MilkTestNZ, Hamilton, NZ

(measurements under the scope of ISO/IEC 17025 accreditation by IANZ; accreditation number 1168)

National Milk Laboratories Ltd., Glasgow, GB

(measurements under the scope of ISO/IEC 17025 accreditation by UKAS; accreditation number 2051)

National Milk Laboratories Ltd., Wolverhampton, GB

(measurements under the scope of ISO/IEC 17025 accreditation by UKAS; accreditation number 2700)

National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Puławy, PL

(measurements under the scope of ISO/IEC 17025 accreditation by PCA; accreditation number AB 485)

Qlip N.V., Zutphen, NL

(measurements under the scope of ISO/IEC 17025 accreditation by RvA; accreditation number L 099)

QSE GmbH (subsidiary of Milchprüfing Bayern e.V.), Wolnzach, DE

State Enterprise Pieno Tyrimai, Kaunas, LT

(measurements under the scope of ISO/IEC 17025 accreditation by Lithuanian National Accreditation Bureau; accreditation number LA.01.106)

State Veterinary and Food Institute, Bratislava, SK

(measurements under the scope of ISO/IEC 17025 accreditation by Slovak National Accreditation Service; accreditation number S-127)

United States Department of Agriculture, Agricultural Marketing Service (USDA-AMS), Dairy Programme - Southwest Federal Milk Marketing Order 126, Carrollton, US

United States Department of Agriculture, Agricultural Marketing Service (USDA-AMS), Dairy Programme - Central Federal Milk Marketing Order 32, Lenexa, US

United States Department of Agriculture, Agricultural Marketing Service (USDA-AMS), Dairy Programme - Appalachian Federal Milk Marketing Order 5, Louisville, US

University of Guelph, Lab Services Division, Agriculture and Food Laboratory, Guelph, CA
(measurements under the scope of ISO/IEC 17025 accreditation by Standards Council of Canada; accreditation number 100)

University of Zagreb, Faculty of Agriculture, Department of Dairy Science, Zagreb, HR

(measurements under the scope of ISO/IEC 17025 accreditation by Croatian Accreditation Agency; accreditation number 1081)

Valacta, Ste-Anne-de-Bellevue, CA

(measurements under the scope of ISO/IEC 17025 accreditation by Standards Council of Canada; accreditation number 99)

Veterinary Faculty – National Veterinary Institute, Naklo, SI

(measurements under the scope of ISO/IEC 17025 accreditation by Slovenska Akreditacija; accreditation number LP-021)

SAFETY INFORMATION

ERM-BD001a and b is intended for laboratory use only. The usual laboratory safety measures apply.

INSTRUCTIONS FOR USE AND INTENDED USE

Reconstitution of the materials shall be performed with reconstitution protocol A or B as outlined below. The protocols need to be strictly followed in order to convert the provided milk powders into milks, which are then either mixed in different ratios to prepare calibration samples (see page 5 a.) or used as such for method performance verification purposes (see page 5 b.).

Reconstitution protocol A

On the day of sample preparation and analysis, allow the samples to warm up to ambient temperature before taking out an aliquot for weighing.

The powder to water ratio to be used is 1 to 9.22. Hence, for 3.00 g milk powder (minimal sample intake per sample replicate), 27.66 mL water (double-distilled or ultrapure/type 1 water quality) shall be added. The water should be pre-warmed to 40 °C before use.

As container for sample preparation, use a clean glass bottle (e.g. borosilicate glass) with a tight screw cap. Alternatively, a sterile plastic bottle with a tight cap can be used.

Add the water to the powder. First gently shake by hand till all powder has come into contact with water (wetting), then add a magnetic stirring rod and stir with medium speed (300 rpm) for 30 min on a stirring hot plate set to 40 °C. Please note that after addition of water and initial gentle shaking by hand, it will take about 10 min until lumps formed will gradually disappear as the powder solubilizes and gets dissolved in the water. Once a homogeneous solution is obtained, take an aliquot and proceed with the analysis without unnecessary delay. Ensure homogenisation and/or mixing before analysis in case the sample/replicate is not analysed right after reconstitution.

Reconstitution protocol B

As an alternative to the reconstitution protocol A described above, protocol B can be used. Investigations at JRC-Geel as well as study results demonstrated equivalence of the two protocols in terms of completeness of reconstitution.

On the day of sample preparation and analysis, allow the samples to warm up to ambient temperature before taking out an aliquot for weighing.

The powder to water ratio to be used is 1 to 9.22. Hence, for 3.00 g milk powder (minimum sample intake per sample replicate), 27.66 mL water (double-distilled or ultrapure/type 1 water quality) shall be added. The water should be pre-warmed to 40 °C before use.

As container for sample preparation, a sterile plastic bottle with a tight cap or a sterile centrifuge tube with a tight cap can be used.

Add the water to the powder. First gently shake by hand till all powder has come into contact with water (wetting). Please note that after addition of water and initial gentle shaking by hand, it will take about 10 min until lumps formed will gradually disappear as the powder solubilizes and gets dissolved in the water. Shake by hand 20 times (inversion mixing), then put bottle for 30 min in a 40 °C water bath, mix again 20 times by hand (inversion mixing), put sample overnight in a cold room or refrigerator (4 °C) for protein rehydration, mix again 20 times by hand (inversion mixing), put bottle for 30 min in 40 °C water bath, final mixing by hand 20 times (inversion mixing), take out aliquot for analysis. Ensure homogenisation and/or mixing before analysis in case the sample/replicate is not analysed right after finalisation of the reconstitution.

The reference materials shall be used for two purposes:

a.) Calibration

The certified value listed on the certificate obtained from the combination of 14 (ERM-BD001a) or 13 (ERM-BD001b) sets of reference method and routine method data sets represents a robust estimate of the true SCC value in those materials. Materials shall be reconstituted as described above and mixed in the quantities shown in Table 1 below. By this, at least 5 calibration levels shall be prepared (equidistant distribution from about 62000 cells/mL and 1166 000 cells/mL) which allow to establish calibration complying with the recommendations of ISO 13366-2.

Table 1. Preparation of calibration samples from the reconstituted materials ERM-BD001a and ERM-BD001b. The concentrations of the intermediate levels and their respective uncertainties are calculated according to IDF Bulletin 469. Concentration values were rounded to the next 1000 cells/mL, uncertainty values were rounded to the next higher 1000 cells/mL. The values in the last two columns assume that 15 mL is a sufficient volume for one calibration level with the appliance used at the customer side. If a larger volume is needed (e.g. 20 mL), 1.33 times higher volumes have to be used in all cases.

Level	Concentration [cells/mL]	Uncertainty [cells/mL]	mL reconstituted ERM-BD001a	mL reconstituted ERM-BD001b
1	1166000	79000	-	15.00
2	890000	59000	3.75	11.25
3	614000	40000	7.50	7.50
4	338000	21000	11.25	3.75
5	62000	6000	15.00	-

Exemplified case in the table (5 times 15 mL needed): for each CRM, 4.5 g powder need to be reconstituted with 41.5 g water to have sufficient milk for preparation of the calibration samples.

b.) Method performance verification

The other certified value listed on the certificate was established from technically valid reference method data sets. This value is to be used for reference method users who want to verify whether their method operates correctly.

The certified value on the certificate described above under a.) can also be used by routine method users who want to verify that their method operates correctly. It shall however be noted that for a dedicated usage of the material, it can either be used for calibration or for method performance verification, but not for both at the same time.

As any reference materials, they can be used for establishing control charts or validation studies.

STORAGE

The materials should be stored at $(-20 \pm 5) ^\circ\text{C}$ in the dark. Care should be taken to avoid any change of the moisture content once the units are open, as the material is hygroscopic. The user should close any bottles immediately after taking a sample.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

LEGAL NOTICE

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(a) make any warranty or representation, express or implied, that the use of any information, material, apparatus, method or process disclosed in this document does not infringe any privately owned intellectual property rights; or

(b) assume any liability with respect to, or for damages resulting from, the use of any information, material, apparatus, method or process disclosed in this document save for loss or damage arising solely and directly from the negligence of the Joint Research Centre of the European Commission.

NOTE

A detailed certification report is available at <https://crm.jrc.ec.europa.eu/>.

A paper copy is obtainable from the Joint Research Centre, Directorate F – Health, Consumers and Reference Materials on request.



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JOINT RESEARCH CENTRE
Directorate F – Health, Consumers and Reference Materials

CERTIFICATE OF ANALYSIS

ERM® - BD001b

MILK		
Cell concentration		
	Certified value ³⁾ [cells/mL]	Uncertainty ⁴⁾ [cells/mL]
Somatic cell count (SCC) ¹⁾	1202000	121000
Somatic cell count (SCC) ²⁾	1166000	79000
¹⁾ As defined in ISO 13366-1. The certified value is the mean value of 13 accepted data sets obtained from ISO 13366-1-compliant measurements. ²⁾ As defined in ISO 13366-1 and ISO 13366-2. The certified value is the mean value of 13 accepted data sets obtained from ISO 13366-1-compliant measurements and 13 data sets randomly selected out of 32 accepted data sets obtained from ISO 13366-2-compliant measurements. ³⁾ Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and with methods of determination referred to in footnotes 1 and 2. The certified value and its uncertainty are traceable to the International System of units (SI). ⁴⁾ The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.		

This certificate is valid for one year after purchase.

Sales date:

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Geel, January 2020

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ANALYTICAL METHODS USED FOR CERTIFICATION

The reference SCC method based on microscopy according to ISO 13366-1 and the routine SCC method based on fluoro-opto-electronic counting according to ISO 13366-2 were used. In some US laboratories, similar methods described in "Standard methods for the examination of dairy products" (chapters 10, direct microscopic SCC and 11.032, electronic SCC) were used.

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SAFETY INFORMATION

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INSTRUCTIONS FOR USE AND INTENDED USE

Reconstitution of the materials shall be performed with reconstitution protocol A or B as outlined below. The protocols need to be strictly followed in order to convert the provided milk powders into milks, which are then either mixed in different ratios to prepare calibration samples (see page 5 a.) or used as such for method performance verification purposes (see page 5 b.).

Reconstitution protocol A

On the day of sample preparation and analysis, allow the samples to warm up to ambient temperature before taking out an aliquot for weighing.

The powder to water ratio to be used is 1 to 9.22. Hence, for 3.00 g milk powder (minimal sample intake per sample replicate), 27.66 mL water (double-distilled or ultrapure/type 1 water quality) shall be added. The water should be pre-warmed to 40 °C before use.

As container for sample preparation, use a clean glass bottle (e.g. borosilicate glass) with a tight screw cap. Alternatively, a sterile plastic bottle with a tight cap can be used.

Add the water to the powder. First gently shake by hand till all powder has come into contact with water (wetting), then add a magnetic stirring rod and stir with medium speed (300 rpm) for 30 min on a stirring hot plate set to 40 °C. Please note that after addition of water and initial gentle shaking by hand, it will take about 10 min until lumps formed will gradually disappear as the powder solubilizes and gets dissolved in the water. Once a homogeneous solution is obtained, take an aliquot and proceed with the analysis without unnecessary delay. Ensure homogenisation and/or mixing before analysis in case the sample/replicate is not analysed right after reconstitution.

Reconstitution protocol B

As an alternative to the reconstitution protocol A described above, protocol B can be used. Investigations at JRC-Geel as well as study results demonstrated equivalence of the two protocols in terms of completeness of reconstitution.

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The powder to water ratio to be used is 1 to 9.22. Hence, for 3.00 g milk powder (minimum sample intake per sample replicate), 27.66 mL water (double-distilled or ultrapure/type 1 water quality) shall be added. The water should be pre-warmed to 40 °C before use.

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Table 1. Preparation of calibration samples from the reconstituted materials ERM-BD001a and ERM-BD001b. The concentrations of the intermediate levels and their respective uncertainties are calculated according to IDF Bulletin 469. Concentration values were rounded to the next 1000 cells/mL, uncertainty values were rounded to the next higher 1000 cells/mL. The values in the last two columns assume that 15 mL is a sufficient volume for one calibration level with the appliance used at the customer side. If a larger volume is needed (e.g. 20 mL), 1.33 times higher volumes have to be used in all cases.

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Exemplified case in the table (5 times 15 mL needed): for each CRM, 4.5 g powder need to be reconstituted with 41.5 g water to have sufficient milk for preparation of the calibration samples.

b.) Method performance verification

The other certified value listed on the certificate was established from technically valid reference method data sets. This value is to be used for reference method users who want to verify whether their method operates correctly.

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As any reference materials, they can be used for establishing control charts or validation studies.

STORAGE

The materials should be stored at $(-20 \pm 5) ^\circ\text{C}$ in the dark. Care should be taken to avoid any change of the moisture content once the units are open, as the material is hygroscopic. The user should close any bottles immediately after taking a sample.

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NOTE

A detailed certification report is available at <https://crm.jrc.ec.europa.eu/>.

A paper copy is obtainable from the Joint Research Centre, Directorate F – Health, Consumers and Reference Materials on request.



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