Development and production of certified reference materials (CRMs) for somatic cell counting in milk

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Feasibility study

- How to enrich somatic cells from raw milk?
- How to stabilize RM preparations?
- Upscaling
- Commutability

- Cell enrichment:

  Microfiltration – resuspension vs. gravity separation – centrifugation – resuspension

  Both worked, microfiltration is more efficient and better reproducible
- Stabilisation of prepared materials

  Freeze-drying vs. spray-drying

  Both are technically feasible although the processes are very different

Feasibility study

- Stabilisation of prepared materials

  Freeze-drying: slower process, most critical part is freezing

  Spray-drying: quick process, cells are exposed to high temperature for a short period of time

- Upscaling issues:

  Envisaged CRM batch size (a few thousand units) cannot be freeze-dried in one cycle, therefore risk of batch-to-batch variability

  Spray-drying only possible outside EC-JRC, can be done in large scale (usual manufacturing process of milk powder)

Feasibility study

- Commutability

  Raw material: raw cow's milk

  Design process to minimize damage to cells as much as possible

  Defatting of milk did not impair the SCC results by laboratories using either microscopic or flow cytometric methods

  Defatted milk: advantage of easier reconstitution

  Low fat content adds to a longer-life stability of the material (no issues with oxidation/rancidness)

  Freeze-drying and spray-drying did not substantially impair the integrity of the cells. Experienced labs employing both microscopic and flow cytometric methods found comparable results for a given sample (raw milk, candidate CRM)

Processing

2 materials, low SCC (ca. 50 000 cells/mL) and high SCC (ca. 1 000 000 cells/mL)

From bulk raw cow's milk to milk powders: NIZO food research, Ede, NL

Pasteurization – cream separation – microfiltration to enrich cells – evaporation for milk concentration – spray drying – dry mixing of spray-dried blank and cell-containing powders to desired SCC levels - shipment of powders to JRC-Geel;

Starting quantity of milk per material: 13 000 litres

Final homogenization, bottling and labelling: JRC-Geel

3-dimensional mixing, manual filling of powders into amber glass bottles under inert atmosphere (glove box), packaging in sets (alumina sachets)

Material code: ERM-BD001

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Processing

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Homogeneity

Measurements with routine method under ISO 17025 accreditation

Per material: 30 samples, duplicate measurements

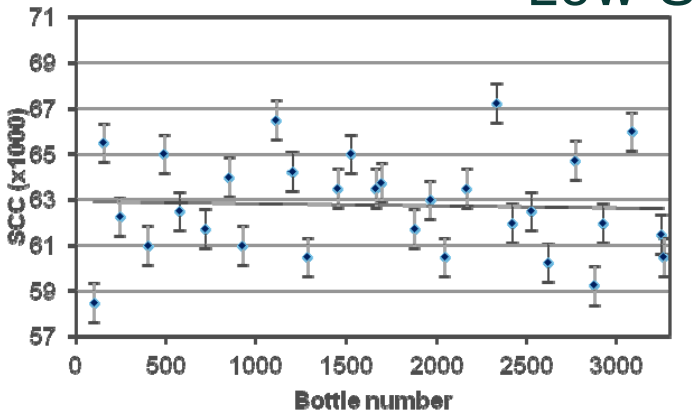
Technical and statistical scrutiny

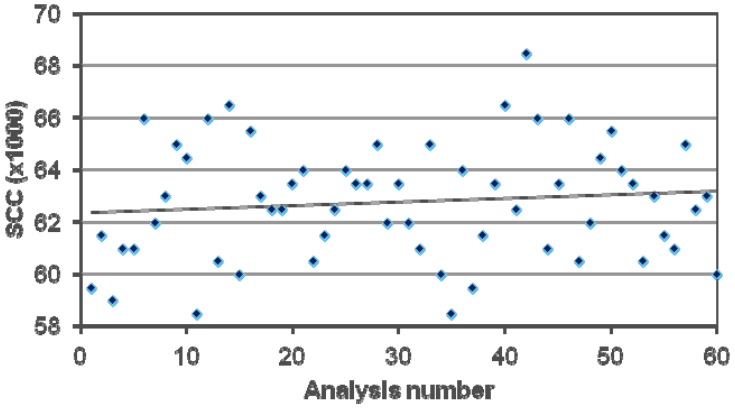
ANOVA to quantify between-unit homogeneity

No outliers, no significant trends for both materials

Uncertainty contributions calculated (potential inhomogeneity)

Low SCC material





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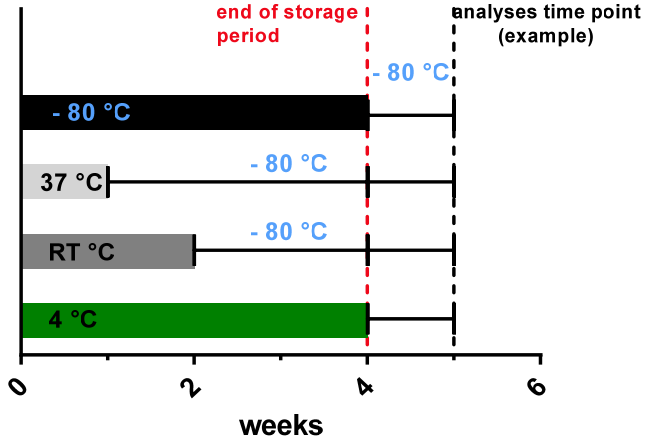
Short- and long-term stability: evaluate dispatch conditions and storage conditions



Routine method under ISO 17025 accreditation

Isochronous study design

Tested temperatures: -20/4/18/60 °C, ref. temp. -70 °C; -20 and 4 °C, ref. temp. -70 °C, respectively

Tested times: 0/1/2/4 weeks and 0/4/8/12 months, respectively

2 (3) units per time/temperatures point, triplicate measurements per unit

Technical and statistical scrutiny

Uncertainty contributions calculated (potential instability during dispatch)

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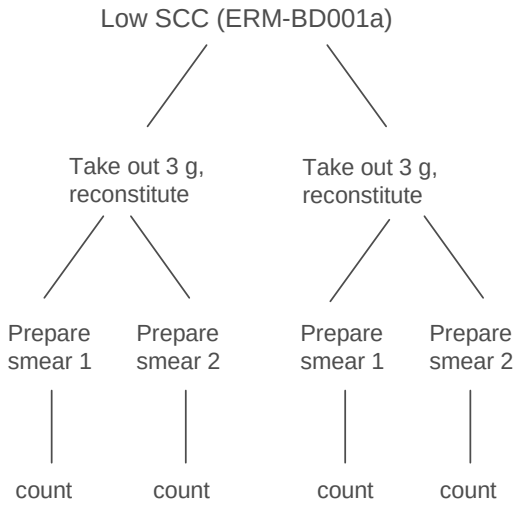
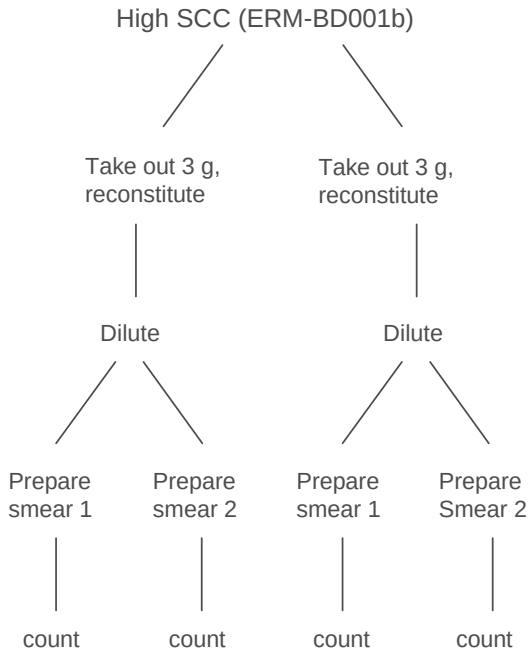
Characterisation study – layouts

Reference method measurements

Day 1





On day 2, the scheme has to be repeated.

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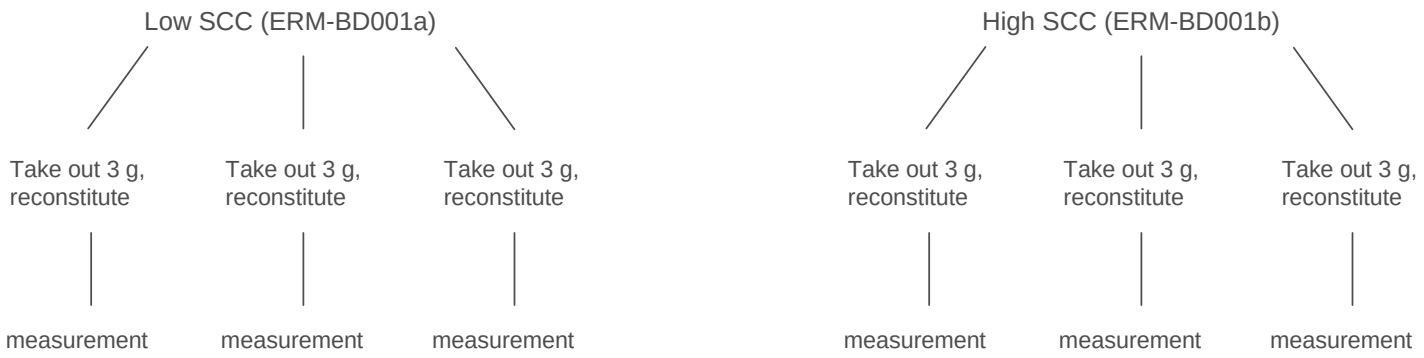
Characterisation study – layouts

Routine method measurements

Day 1



On day 2, the scheme has to be repeated.

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Characterisation

32 laboratories from 16 European and 5 non-European countries

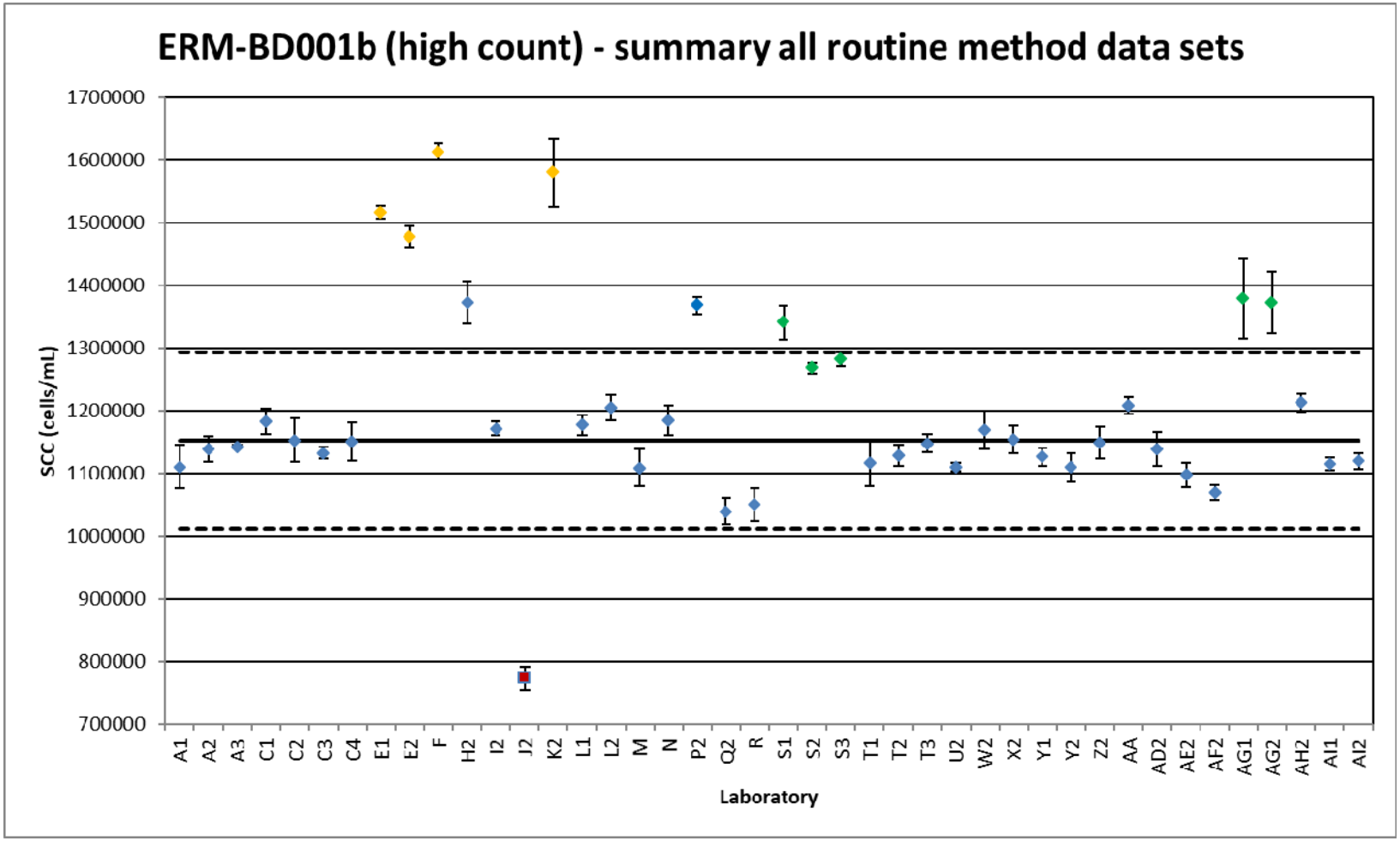
Provided with the samples:

- Reconstitution protocol
- Detailed instructions for work (text and graphics)
- Method questionnaire to fill in method details
- Results sheet to fill in results

19 reference method data sets and 42 routine method data sets obtained for each material

Characterisation - example

Routine method results



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Value assignment

Guidance in ISO 13366-2 of how to prepare calibration samples and assign a reference value to those

Use mean of reference method data sets. If instrumental data is available and the mean values of reference and routine data do not differ more than 10%, data can be combined, but the weighing of the reference data needs to be at least 50%.

Decision to assign 2 certified values per material:

1.) Reference data only (n=14 and 13, respectively)

2.) 14 (13) reference data combined with 14 (13) routine data
Only routine data with a slope between 0.95 to 1.05 were taken into account
From the n=32 routine data sets each with a slope around 1,
14 (13) were randomly picked

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Certified values, uncertainties, traceability

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 randomly selected data sets 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.

Intended use of the materials

1.) Assessment of reference method performance

2.) a.) Calibration of routine method
b.) Assessment of routine method performance

Note: No calibration and method performance assessment in the same instance!

3.) QC-charts, method validation

4.) Assign values to secondary/in-house RMs

More information

EC-JRC reference material catalogue: https://crm.jrc.ec.europa.eu/

Direct link to ERM-BD001: https://crm.jrc.ec.europa.eu/p/q/erm-bd001+/ERM-BD001-MILK-POWDER-somatic-cell-count-SCC/ERM-BD001

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Thank you

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