Milchprüfring Bayern e.V.

Suitable SCC Reference Materials for Flow Cytometry Counting

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Background of mpr and mission

mpr is the largest raw milk testing organization in Germany, processing

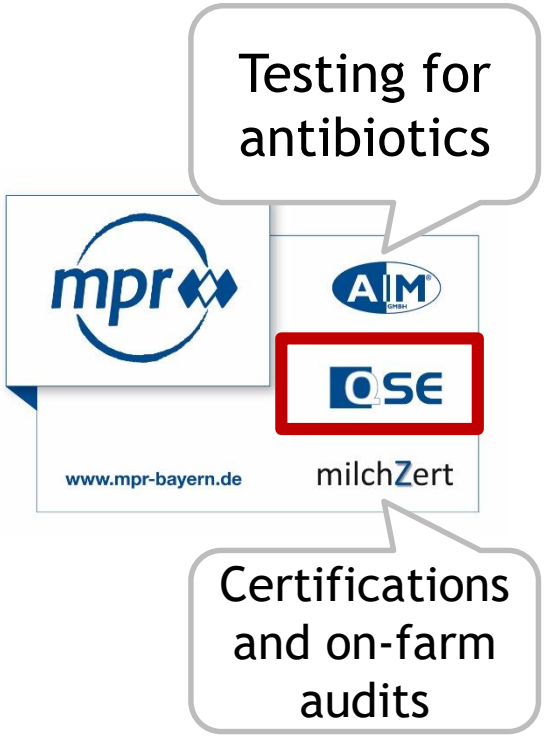
- 5 Mio. samples per year from 30.000 farms for quality testing and
- 9,2 Mio. samples per year from 960.000 cows in the DHI system.

Together with other lab services, this sums up to more than **16 Mio. SCC results** per year validated and reported to farmers, dairies and other organizations.

Background of mpr and mission

mpr has founded three affiliate companies to create and utilize specific knowledge, covering the main areas of mpr mission

- to check and promote raw milk quality
- to promote food quality, thus serving consumer protection.



DIN EN ISO/IEC 17034:2017 (since Feb2019) ➔ reference material producer

DIN EN ISO/IEC 17043:2010 (since Dec2017) ➔ proficiency test provider

Since 1998 ➔ Development of frozen raw milk reference materials for mpr´s own needs

Since 2006 as **QSE GmbH** ➔ product portfolio covers many different reference materials, special standards and services including proficiency tests for quality assurance in dairies and milk laboratories

customers in **45 countries** (growing)

QSE specialty ➔ frozen and lyophilized long-term stable certified reference materials

- ✓ no preservatives
- ✓ long-term stability (≥ 2 years from production date)
- ✓ storage temperature ≤ -20 °C / 6 ± 2 °C
- ✓ simple and quick application (< 60 min)
- ✓ independently validated, highly secured reference values
- ✓ detailed certificate of quality

Available materials are…

- ✓ Cow`s / Goat`s Raw Milk
- ✓ Pasteurized Milk / Skimmed Milk / Cream
- ✓ Whey and Special Standards
- ✓ Characterized for different parameters and concentrations:
  Fat, Protein, ……, SCC, ……, PAG, fatty acid profile

Crucial characteristics for RMs

- Homogeneity
- Target value for the analyte(s) – „reference value“
- Stability (during shelf life)

Homogeneity (of filling)

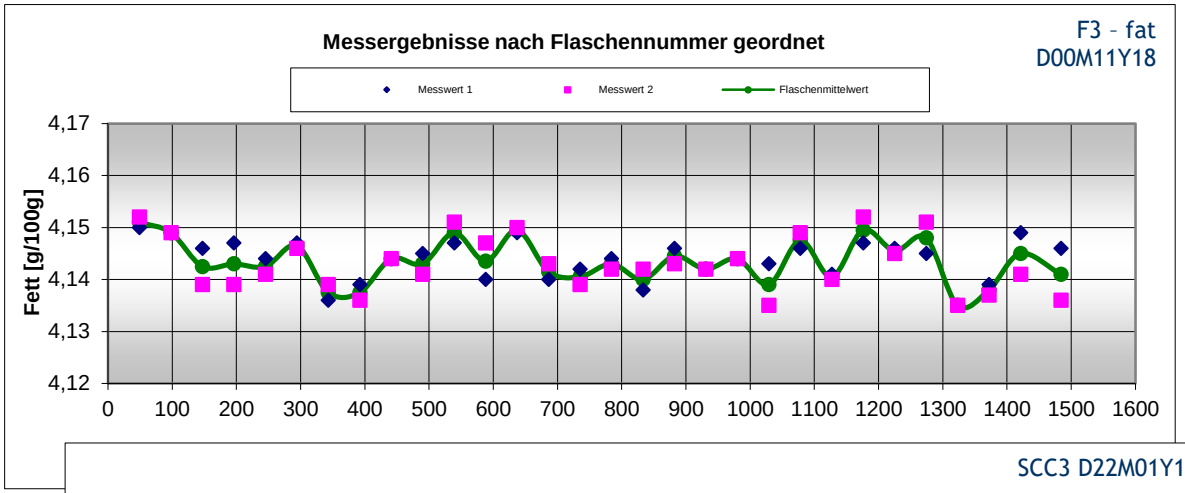
- Take 10 – 30 samples of the whole batch
- Do repeated measurements in random order

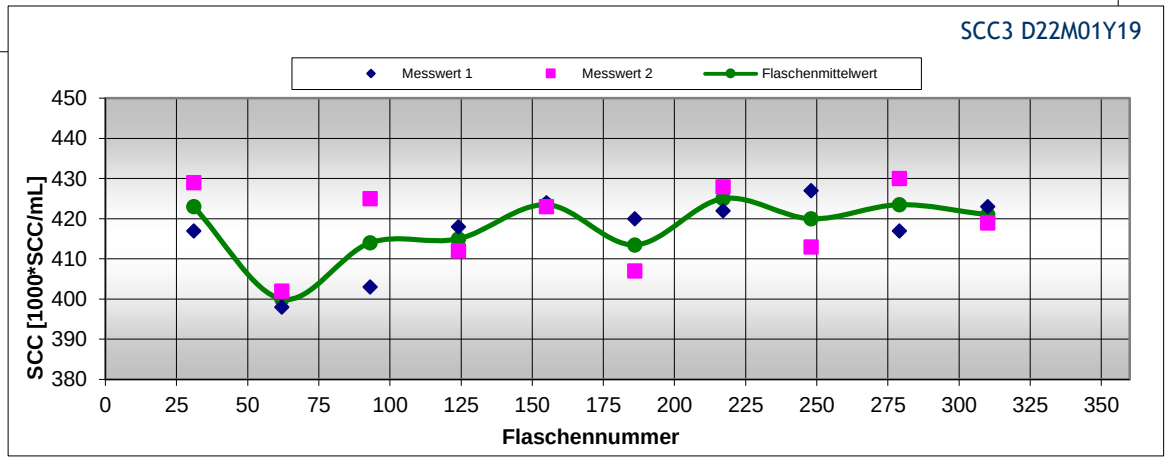
variance between bottles = total variance – analytical variance

- **Visual** evaluation
- **Statistical** evaluation

  test for equality of variances between the bottles (Cochran-Test)

  homogeneity is sufficient, if uncertainty between packaging units is negligibly small compared to uncertainty of reference method (F-Test)





Characterization ➔ target value

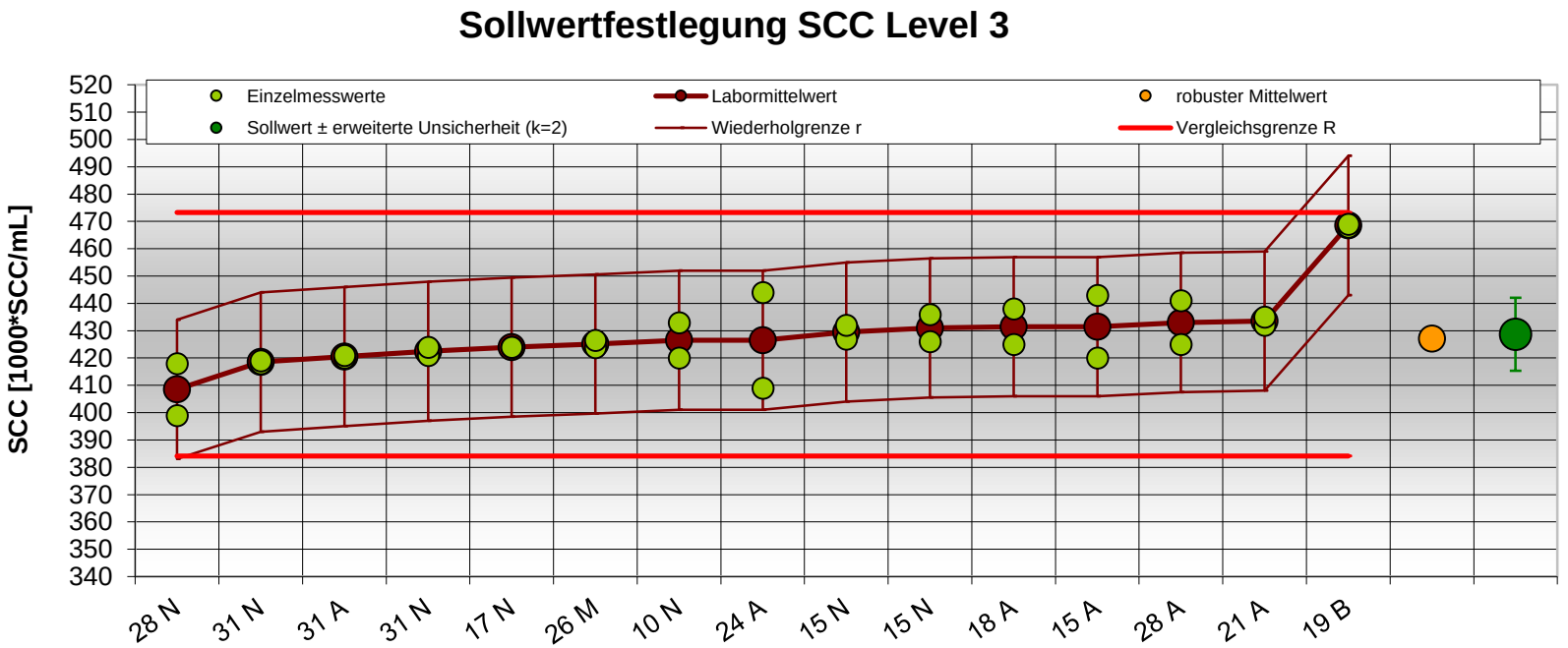
- Measured with recognized **reference method**(s)
- in # **accredited reference laboratories**
  (number of labs depends on product and parameter)

target value / “reference value” =
arithmetic mean of all statistically verified measurements

expanded uncertainty ➔ $U_{crm} = 2 \cdot u_{crm} = 2 \cdot \sqrt{(u_{hom}^2 + u_{char}^2}$

expanded uncertainty for SCC ➔ $U_{crm} = 2 \cdot u_{crm} = 2 \cdot \sqrt{(u_{hom}^2 + u_{char}^2 + u_{lstab})}$

Characterization ➔ SCC3 D22M01Y19



Example ➔ Reference values

from the Data Sheet SCC 1-5 D22M01Y19

Reference values:

The somatic cells were analyzed in multiples in nine different ISO / IEC 17025 accredited laboratories using automated fluorescence optical counting and one ISO / IEC 17025 accredited laboratory using microscopic counting. The reference value is the arithmetic mean of all statistically checked measurement results. Accuracy of the reference value was ensured by comparison with the robust mean.

The expanded uncertainty (k=2) was determined according to ISO Guide 35, taking into consideration the uncertainty packaging units, the uncertainty of characterization and the uncertainty of long-term stability included.

The metrological traceability is based on the above-named reference method.

Parameter	Method	Product Appellation	Number of results	Reference value ± expanded uncertainty (k=2)	
Somatic Cells	Flow Cytometry / Microscopy	SCC1	30	130.000 ± 4.900	SCC/mL
		SCC2	30	265.000 ± 9.000	
		SCC3	30	429.000 ± 13.300	
		SCC4	30	766.000 ± 19.200	
		SCC5	30	1.081.000 ± 24.300	

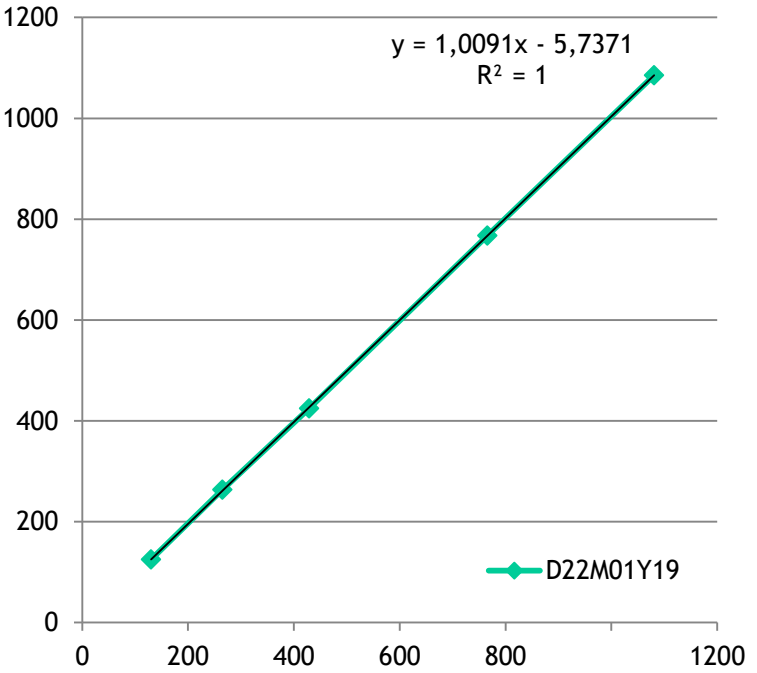
Evaluation of Characterization

- detailed **checks** and **statistical evaluation** of the measurements
  → test for normal distribution (Shapiro-Wilk-Test)
  → test for equality of variances between the labs (Cochran-Test)
  → outlier-test (Grubbs-Test)
- application of **sensitive** and **robust statistical methods***
- comparative measurements with flow cytometers **(„FC prediction value“)** for internal control
- graphical representation and **visual evaluation**
- **test calibrations**

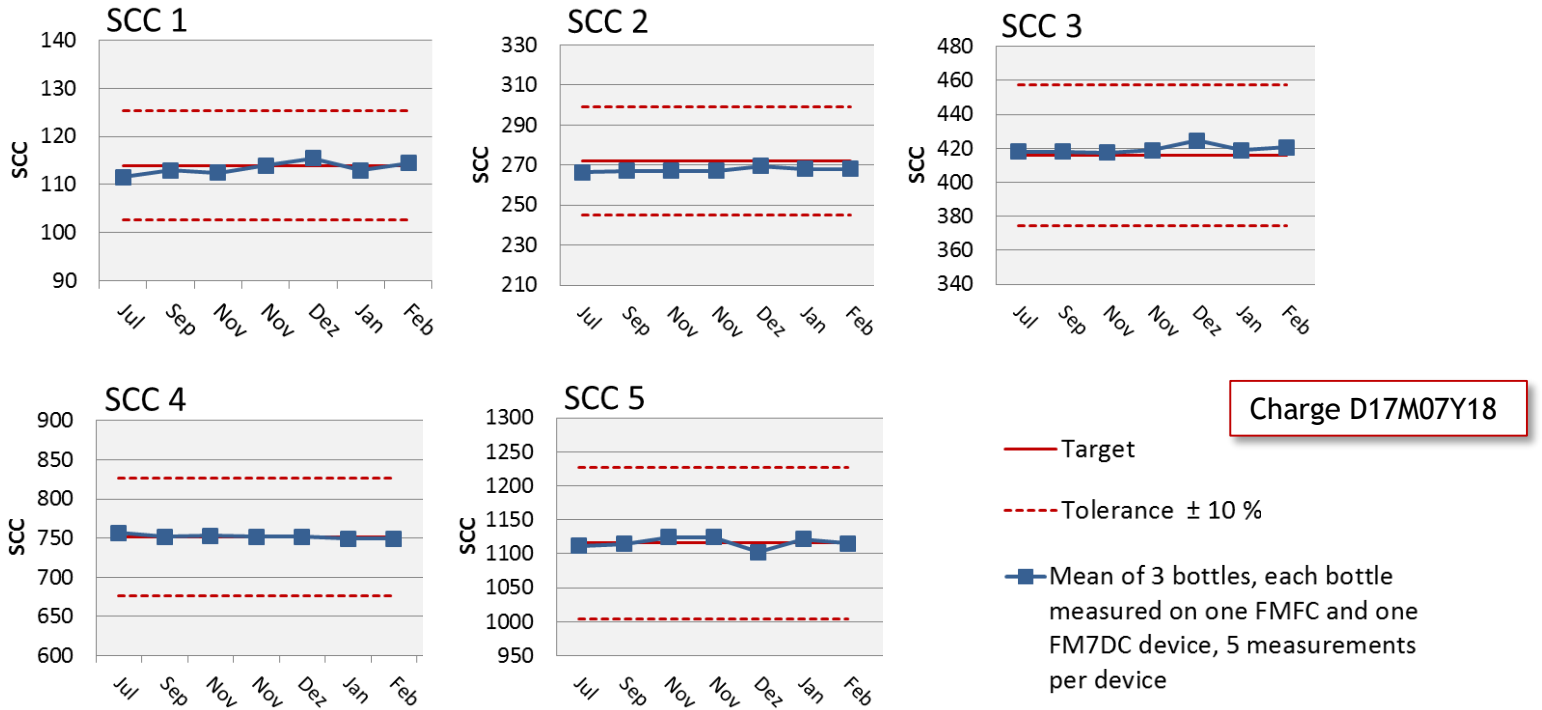
* robust statistics according to ISO 5725-5, algorithm A

Test calibration SCC 1 – 5 (frozen)

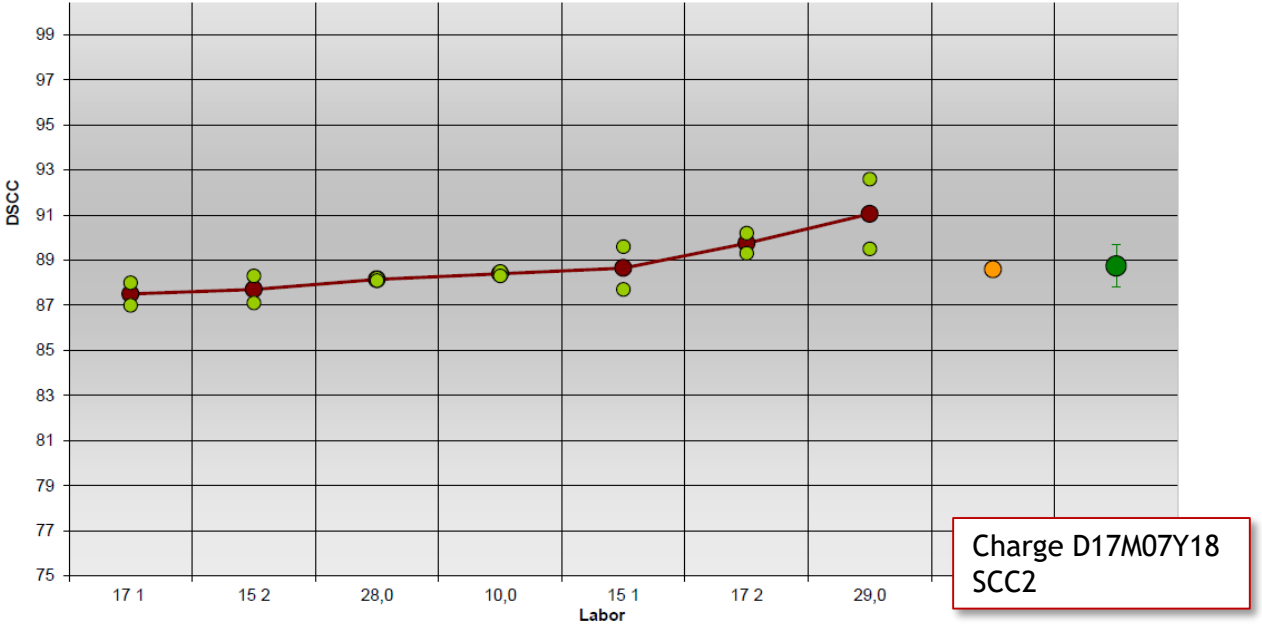
	1	2	SCC3	4	5
10 Results each sample	122	272	431	761	1106
	130	259	414	773	1072
	127	260	420	769	1084
	125	264	434	796	1109
	124	249	411	764	1045
	123	275	414	756	1091
	130	254	433	779	1104
	125	277	444	743	1086
	121	279	433	773	1082
	126	248	415	758	1077
Average Value	**125**	**264**	**425**	**767**	**1086**
Standard Deviation	3,057	11,55	11,4	14,47	19,02
VK	**2,4%**	**4,4%**	**2,7%**	**1,9%**	**1,8%**
Own limits VK	6,0%	6,0%	4,0%	3,0%	3,0%
Deviation. MW-Soll	-5	-1	-4	1	5
% Deviation MW-Soll	-4	0	-1	0	0
Reference Value	130	265	429	766	1081



New SCC standard – stability studies



Characterization for DSCC



Thank you for listening!!!

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