Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Departement of Economic Affairs DEA
Agroscope Liebefeld-Posieux Research Station ALP

Production and Characterization of reference material by ALP

Thomas Berger, Sandra Brunner, Lotti Egger

02. October 2008

History of ALP Somatic Cell Count Standard (SCCS)

Beginnings

- approx. 1984 cooperation of ALP and VetSuisse faculty to produce a SCCS (Schällibaum, Limacher, Merminod)
- 1986 extraction of thymocytes and (because of BSE) later leucocytes from blood of young bulls by Bommeli corporation, preparation of ALP SCCS for Swiss raw milk control laboratories; reference value based only on ALP value
- approx. 1995 first customers in other countries
- 2002 more customers in other countries

History of ALP SCCS

Improvements

- 2004 reference value based on an international characterization
- 2004/2005 stability problems, transfer of the cell extraction to ALP, improvement of the procedure

  (Meyer, Bühlmann, Raemy, Brunner, Aebi)
- 2008 optimization of fixation procedure

  (Egger, Raemy, Brunner)

History of ALP SCCS

Use of ALP SCCS

- Germany
- Austria
- Switzerland
- Italy
- Croatia

- Chile

Preparation of SCCS

Main steps

- Fresh blood from young bulls containing 10% anticoagulant solution
- Filtration
- Centrifugation, removal of blood serum
- First lysis of red blood cells with EDTA, centrifugation after addition of PBS
- Resuspension of the sediment with PBS
- Second lysis as before
- Centrifugation
- Resuspension of the sediment with PBS
- Third lysis as before
- Centrifugation

Preparation of SCCS

Main steps

- 2 further wash steps with PBS
- Centrifugation
- Resuspension of leucocytes in skim milk containing fixation solution (10% ethanol, Bronopol, and polypropylen-glycol)
- Addition of a defined volume of leucocyte suspension to UHT milk containing Bronopol and polypropylen-glycol to obtain a high and a low standard

Use, quantities and price

- The ALP Somatic Cell Count Standard (SCCS) is used for the evaluation of comparability, reproducibility and traceability of automated cell count measurements
- it covers the range from 150'000 up to 450'000 cells/mL on two levels
- quantities: 7, 20, 27 and 40 mL
- frequency: monthly, bimonthly, quarterly
- Price: CHF -.45/mL incl. postage

thomas.berger@alp.admin.ch

Characterization of reference value

- based on DMSCC and ASCC measurements
- using a pool of experienced international laboratories, including:
  - MCC-Vlaanderen, Lier (BE)
  - BfEL/Max-Rubner-Inst, Kiel (DE)
  - mpr-BW, Kirchheim (DE)
  - mpr-BY, Wolnzach (DE)
  - Greek Dairy Org., Ioannina (GR)
  - Greek Dairy Org., Larisa (GR)
  - Greek Dairy Org., Patras (GR)
  - Greek Dairy Org., Pella (GR)
  - ICBA, Caesaria (IL)
  - AIA Isl, Roma (IT)
  - SV Südtirol, Bolzano (IT)

Characterization of reference value

- Qlip, Zutphen (NL)
- RIKILT, Wageningen (NL)
- mpr-OÖ, Ried (AT)
- University of Vienna/ Veterinary Dep. (AT)
- LNIV; Lisboa (PT)
- ALP, Liebefeld (CH)
- Suisselab AG, Zollikofen (CH)
- LA-Santander, Santander (SP)
- CMIO C.T.LAB, Nicosia (CY)

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Agroscope Liebefeld-Posieux Research Station ALP

Characterization of SCC reference material

Name of Laboratory:	LABORATORIO STANDARD LATTE		
Samples arrival date:	03.09.2008	**slope:**	
State of the samples at arrival:	GOOD	**intercept:**	
Date of launching of the analyses:	04.09.2008		

Automated measurement

Type of equipement: BENTLEY SOMACOUNT 150

Raw data	Sample low 1	Sample low 2	Sample low 3	Sample high 4	Sample high 5	Sample high 6		low	high
Measurement 1	232	234	219	507	505	518			
Measurement 2	219	229	221	507	520	516			
Measurement 3	229	223	230	498	517	511			
Measurement 4	229	218	225	501	496	511			
Measurement 5	220	225	225	495	510	508			
Mean value	**226**	**226**	**224**	**502**	**510**	**513**	Total mean	225	508
Standard deviation	6	6	4	5	10	4	Total standard deviation	5	8
Coefficient of variation %	2.6	2.7	1.9	1.1	1.9	0.8	Total CV %	2.3	1.6

Microscopic measurement

Raw data sample "high"	Operator 1: countings 1st	2nd	3rd	Operator 2: countings 1st	2nd	3rd		high
Film a	521							
Film b	520							
Mean value	**173**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	Total mean	521
Standard deviation	16	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Total standard deviation	1
Coefficient of variation %	9.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Total CV %	0.1

Raw data sample "low"	Operator 1: countings 1st	2nd	3rd	Operator 2: countings 1st	2nd	3rd		low
Film a	161							
Film b	184							
Mean value	**173**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	Total mean	173
Standard deviation	16	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Total standard deviation	16
Coefficient of variation %	9.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Total CV %	9.4

- Procedure: the labs receive samples by normal post and an Excel evaluation - sheet by email
- The measurement results are returned by email within 14 days

Characterization of reference value

- Performance of statistical treatment of data including: outlier test, mean value, standard deviation, coefficient of variation, repeatability, reproducibility, Mandel statistics and graphs

A) Routine Instruments, all data, NOT NORMALIZED BY SLOPE-INTERCEPT

Routine Instruments, Results and Group Means in SCC/µl

CODE	COUNT	L_1	L_2	L_3	L_MEAN	H_1	H_2	H_3	H_MEAN
T_01	1	274	261	268		467	489	488	
T_01	2	266	257	274		492	478	480	
T_01	3	263	276	267		460	482	498	
T_01	4	268	278	266		468	467	465	
T_01	5	274	253	265	267.3	452	475	469	475.3
T_02	1	244	250	254		457	466	477	
T_02	2	250	258	234		458	465	445	
T_02	3	243	254	248		471	453	456	
T_02	4	247	249	249		454	448	453	
T_02	5	238	266	248	248.8	453	436	461	456.9
T_03	1	267	258	247		454	448	478	
T_03	2	248	249	265		458	467	468	
T_03	3	272	258	243		445	451	450	
T_03	4	256	265	264		461	438	462	
T_03	5	257	268	243	257.3	470	456	451	457.1
T_04	1	258	250	247		442	449	426	
T_04	2	256	256	249		437	476	433	

Routine Instruments, Basic Statistics, Instrument Results sorted by Means
Standard ZZS_07C_L, SCC/µl

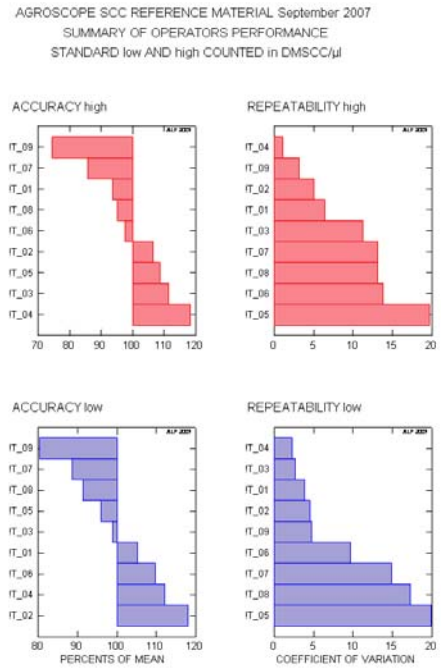
T_CODE	N	MEAN	SD	CV	Z-VAL	PERCENTS
T_09	15	190.73	12.07	0.06	-2.83	75.17
T_14	15	233.20	7.25	0.03	-0.98	91.91
T_13	15	240.67	32.84	0.14	-0.65	94.85
T_02	15	248.80	7.72	0.03	-0.30	98.06
T_11	15	254.73	5.69	0.02	-0.04	100.39
T_03	15	257.33	9.49	0.04	0.08	101.42
T_04	15	258.13	10.35	0.04	0.11	101.73
T_10	15	258.40	10.32	0.04	0.12	101.84
T_07	15	260.20	10.73	0.04	0.20	102.55
T_15	15	260.73	25.70	0.10	0.23	102.76
T_05	15	265.67	4.67	0.02	0.44	104.70
T_01	15	267.33	7.09	0.03	0.51	105.36
T_06	15	269.67	11.79	0.04	0.62	106.28
T_08	15	272.93	8.56	0.03	0.76	107.57
T_12	5	295.00	7.91	0.03	1.72	116.26

Characterization of reference value

- reference value is the mean value of the AFEMA laboratories → observed reproducibilities are usually better in equilibrated measurement networks
- see ISO/IDF action for a reference system for somatic cell counting

SCC/µl

LEVEL	LABS	N	MEAN	SL	Sr	SR	RSDL	RSDr	RSDR	r	R	R/r
ZZS_07C_L	15	215	253.74	21.34	13.88	25.45	8.41	5.47	10.03	39.27	72.00	1.83
ZZS_07C_H	15	215	461.67	27.43	15.32	31.42	5.94	3.32	6.80	43.32	88.86	2.05

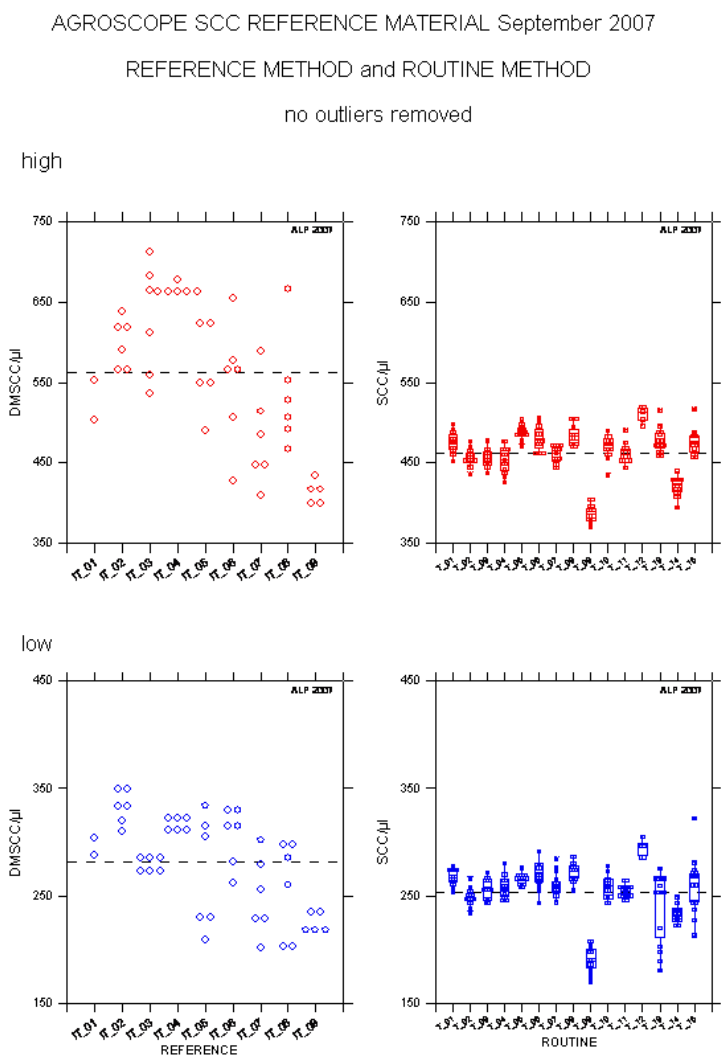


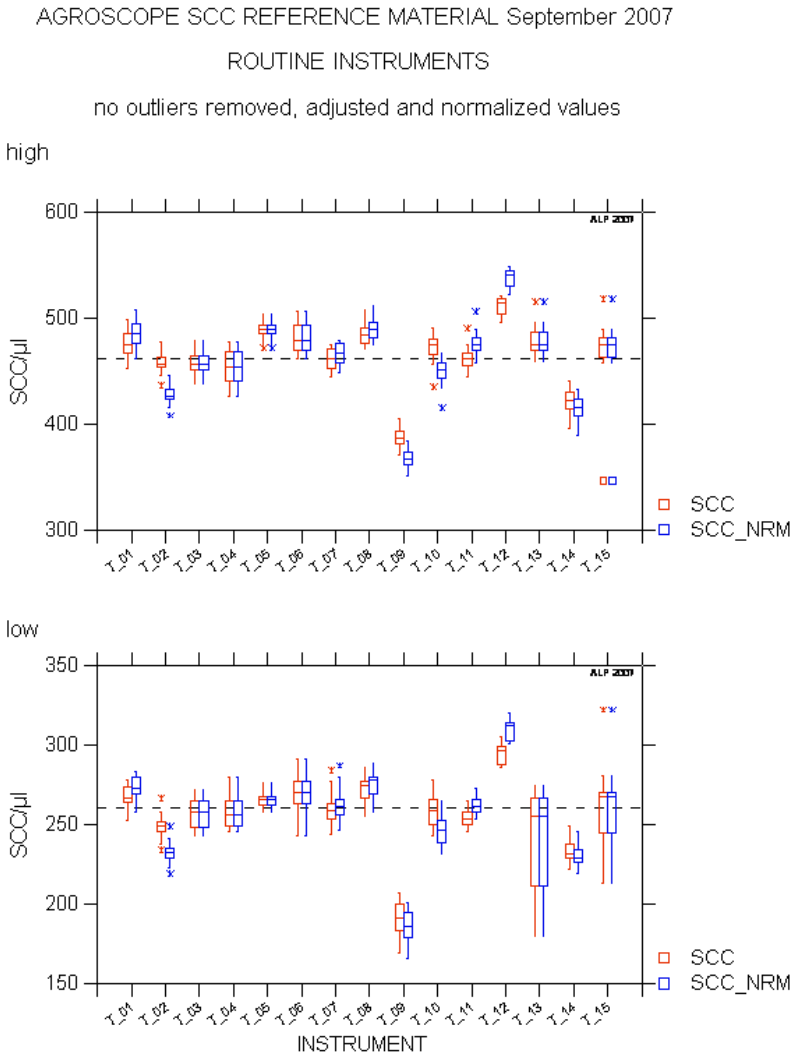
B) Routine Instruments, AFEMA data

SCC/µl

LEVEL	LABS	N	MEAN	SL	Sr	SR	RSDL	RSDr	RSDR	r	R	R/r
ZZS_07C_L	9	150	260.91	5.23	12.02	13.11	2.01	4.61	5.02	33.99	37.08	1.09
ZZS_07C_H	10	150	468.90	10.44	16.35	19.40	2.23	3.49	4.14	46.24	54.87	1.19

Characterization of reference value





Characterization of reference value