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AN EXPERIMENTAL COMPARISON OF METHODS FOR SOMATIC CELL COUNT DETERMINATION IN MILK OF VARIOUS SPECIES OF MAMMALS

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Abstract

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Somatic cell count (SCC) is important foodstuff, hygienic and health indicator of milk and animal mammary gland. The goal of this paper was to evaluate an ability of chosen methods to reach the SCC reliable results in various biological kinds (species) of milk. The various methods of SCC determination were compared in cow (CM), goat (GM), sheep (SM) and human (HM) milk: direct microscopy (DM); fluoro-opto-electronic (Fossomatic 90; Foss); fluorescent (DCC; De Laval). Used methods had cow milk calibration basically. The DM, Foss and DCC result relations about SCC were very close, mostly ≥ 0.92 ($P < 0.001$) for CM, GM and SM. In CM the regression equations between methods were near ideal form $y = 1x + 0$. The mean differences SCC data sets between mentioned methods were small for CM, larger for SM and HM and the largest for GM. It is possible to convert all DCC results in SM, HM and GM to DM or Foss method. The conversion equations were stated from DCC: to DM in cow milk $y = 1.1293x - 5.5029$; to Foss in goat milk $y = 3.603x - 3171.4$; to Foss in sheep milk $y = 1.3805x - 18.149$; to Foss in human milk $y = 2.6246x + 158.63$. Assesment of conversion equations should be individual laboratory event. Results had relatively good correspondence among DM, Foss and DCC for SCC determination in CM, GM, SM and HM for milk quality control. DCC had lower results in small ruminants as compared to Foss calibrated on CM using DM. DCC in HM had lower results as Foss adjusted by CM at good correlation (0.84; $P < 0.001$).

cow, sheep, goat, human milk, somatic cell count (SCC), direct microscopy, fluoro-opto-electronic method, fluorescence method, relationship

Somatic cell count (SCC) in milk is important food-hygienic (bulk samples) and also health (individual samples) indicator of animal mammary gland. Somatic cells are mostly leucocytes in milk (nucleus cells) which show on actual state of physiological and pathological response of animal defense system with respect to possible mammary gland infection. The SCC values show on milk secretion disorders occurrence. Therefore the SCC determination serves over whole world to control of milk food chain quality in dairying and human nutrition from beginning of this succession. Critical quality values of SCC are limited by legislation for milk of some animal species.

For cover of above mentioned facts more analytical methods are existing which are based on various principles, standardized and also unstandardized. Beside reference direct optical microscopy method (CSN EN ISO 13366–1 (DM)) there are also instrumental methods. Electronical counting of firm particules (somatic cells fixed by formaldehyde) during their passage throught defined electrical field in capillary was previous procedure (so called Coulter Counter method). Today other procedures such as fluoro-opto-electronic counting (CSN EN ISO

13366–2) of stained (ethidiumbromid) somatic cell nuclei (DNA) which emit red emission after lighting which is registered electronically by disc rotation (DR, disc rotation; Fossomatic) method or more modern flow cytometry (FC, flow cytometry; for instance Fossomatic – Foss Electric, Denmark (Foss), Somacount – Bentley Instruments, USA or Somascope – Delta Instruments, The Netherlands) are used preferably. Also various modifications of viscosimetric methods (Wisconsin Mastitis test or Ruakura (New Zealand) rolling ball viscosimeter (Hanuš *et al*., 1993d)) on the principle of so called Schalm reaction (reaction among DNA, milk proteins and added detergent with creation of gel and its density increase at SCC rise; Hejlíček *et al*., 1987) are disposable for estimation of cell quantity as well. There are above all Schalm California mastitis test in modification known as Mastitis tets NK in the Czech Republic. Recently a further instrumentation (Chemometec (2004), NucleoCounter SCC–100 and De Laval (2006), DCC cytometer, fluorescent method principle (DCC)) is appearing, especially for direct advisory service purposes at fast and operative monitoring of udder health in animal stables (Castro *et al*., 2008; Sánchez–Macías *et al*., 2008). All these indirect instrumental methods of SCC determination should be calibrated or adjusted in other way according to direct microscop SCC determination results. Differences in result reliability regarding biological kind of milk can exist here.

The row of papers was interested in methodical support of reference standard preparation for somatic cell count (SCC) determination in milk, especially in cows (Szijarto and Barnum, 1984; Lintner *et al*., 1984; Arndt *et al*., 1991; Heeschen *et al*., 1994; Aebi and Bühlmann, 2000a, b; Baumgartner *et al*., 2000). Also whole row of papers was interested in statistical evaluation of milk analytical result reliability in laboratory networks on national and international level (Vines *et al*., 1986; Grappin, 1987, 1993; Valenberg, 1990; Arndt *et al*., 1991; Leray, 1993, 2006, 2010; Wood, 1994; Heeschen *et al*., 1994; Golc–Teger, 1997; Wood *et al*., 1998; Baumgartner *et al*., 2000; Fuchs, 2000; Coveney, 2001; Feinberg and Laurentie 2006; Hanuš *et al*., 2006, 2007; Hering *et al*., 2008; Říha *et al*., 2008 – proficiency testing (PT)).

SCC determination in other biological kinds of milk in other farm animals (sheep, goats, buffalos, camels, horses or asses), mammals respectively, than in cows has growing importance. The main reason is an increase of herds of various animal species (Nicolas *et al*., 2008; Pellegrino and Rosi, 2008; Marchand *et al*., 2008) and also spectrum of milk food (for instance functional food) and its market. It means spreading of milk market and growth of an importance of food safety and consumer health control. The aim of this work was to examine pertinence of chosen analytical procedures to product reliable SCC results in various kind of milk which can methodically extend possibilities of control of hygiene standards and quality in dairying but especially in an animal health state.

MATERIAL AND METHODS

Model sample files of various biological kinds of milk

Human milk samples were analysed as unpreserved. Other mammals species milk samples (ruminants) were preserved (0.02%; Pettipher and Rodrigues, 1982; Ardö, 1982; Kroger, 1985; Hanuš *et al*., 1992a, b; Genčurová *et al*., 1993a, 1994; Benda, 1995) by peletted bronopol (2–brom, 2–nitro, 1, 3 propandiol; Control System D&F Microtabs, England). First and also second samples were analysed either after cold transport and storage (< 6 °C; according to Szijarto *et al*., 1990 and Sojková *et al*., 2009) or frozen preservation (–21 °C), according to conditions, however always in accordance with standard procedure. Previous papers (Hanuš *et al*., 2002, 2008; Baumgartner and Landgraf, 2004) demonstrated also possibility of milk sample frozen preservation as usable procedure before analyse for obtaining reliable SCC results. Experimental comparison covered 1.5 lactation period of small ruminants by realization time and in this way it took 1.5 year.

I) cow milk (CM): reference (for PT), modified, mixed bulk and individual milk samples (n = 20, in two tests A and B) species *Bos primigenius* f. *taurus* (L), breeds Czech Fleckvieh and Holstein. Native and frozen preserved samples, native samples were chemical preserved. The used methods of SCC determination were DM (proficiency testing, Fig. 1 with good result), Foss and DCC.

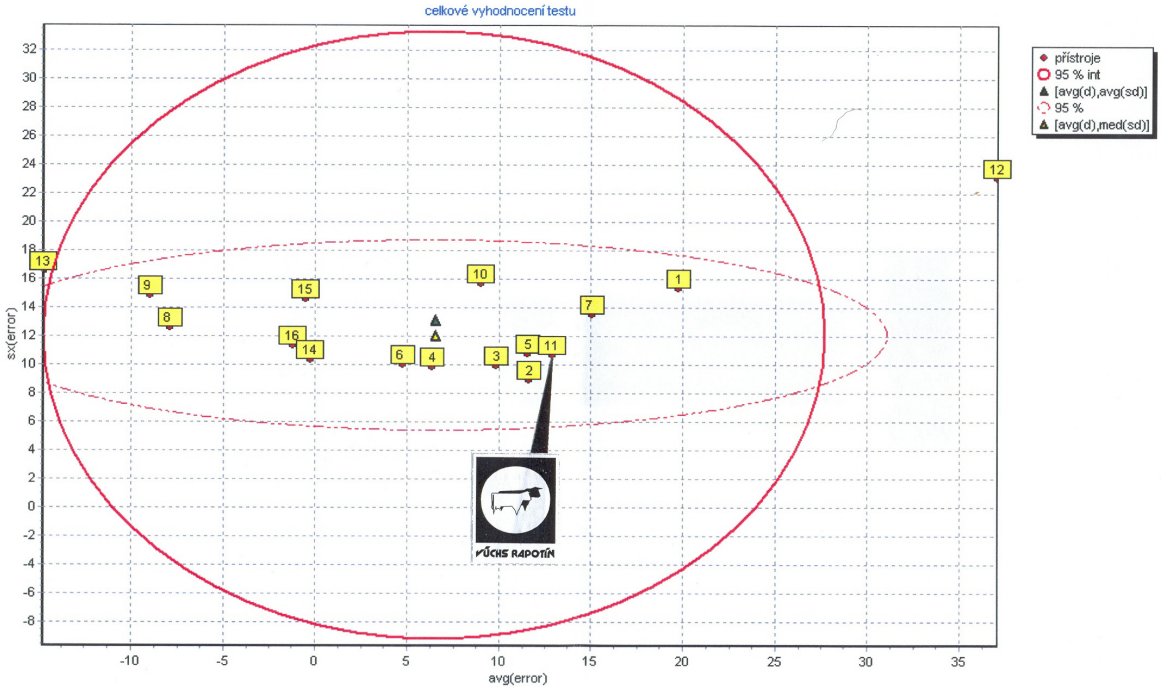
II) goat milk (GM): bulk milk samples (n = 30 samples, 3 tests A, B and C) from small count of animals (from 4 to 6 animals in one sample) species *Capra aegagrus* f. *hircus* (L), breed White short haired, from morning milking. Native milk samples were subsequently chemical preserved and also frozen preserved. Used methods of SCC determination were Foss and DCC.

III) sheep milk (SM): bulk milk samples (n = 40 samples, 4 tests A, B, C and D) from small count of animals (from 4 to 6 animals in one sample), species *Ovis aries* (L), greed Tsigai, from morning milking. Native milk samples were subsequently chemical preserved and also frozen preserved. Used methods of SCC determination were Foss and DCC.

IV) human milk (HM): individual milk samples (n = 54 samples, 1 test A), species *Homo sapiens sapiens* (L), originated from primipar mothers in age from 23 to 27 years. Samples were taken during 1.5 year, from 2nd to 47th lactation week, always in morning hours after 12 hunger hours. Whole volume of milk was exhausted from breast which was not suckled last time. The sample obtained in this way was frozen preserved before analyse. Used methods of SCC determination were Foss and DCC.

Examined analytical methods for somatic cell count determination in milk

The direct microscopy method (DM) was used for counting of stained somatic cells (CSN EN ISO



1: *Regular proficiency test graph for somatic cell counters (fluoro–opto–electronic technology by disc rotation (DR) or flow cytometry (FC)) in cow milk in the Czech Republic (n = 16)*
(SVI Prague, NRL-M, authorized software SomaRing, AgriResearch Rapotin, Říha *et al.*, 2008; used apparatus Fossomatic 90 in National Reference Laboratory for Raw Milk in methodical comparison (official proficiency testing) from period of method comparison of SCC determination on various biological kinds of milk is marked)

13366–1) at SCC determination in CM reference standards for proficiency testing (PT) purposes. Milk samples from all species (CM, GM, SM, HM) were analysed on SCC using fluoro–opto–electronic method in DR type (Foss) on Fossomatic 90 (Foss Electric, Denmark) apparatus with regard to previous methodical knowledge (Grappin and Jeunet, 1974, 1975; Coleman and Moos, 1989; Genčurová *et al.*, 1993b; Hanuš *et al.*, 1993a, b, c, 2002, 2007, 2009) and according to CSN EN ISO 13366–2. This apparatus was included in proficiency testing (State Veterinary Institute, Prague) for SCC determination regularly three times a year. The results were regularly in order (Fig. 1). The extended combine result uncertainty (Suchánek *et al.*, 1999) of measurement was estimated in accredited laboratory about ± 9.3% for SCC ≤ 900 10^3.ml^{-1}. The mentioned SCC result assurance was in accordance with approaches which were published by Valenberg (1990), Arndt *et al.* (1991), Grappin (1993), Leray (1993), Heeschen *et al.* (1994), Wood (1994), Golc–Teger (1997), Wood *et al.* (1998), Baumgartner (2000), Fuchs (2000), Aebi and Bühlmann (2000a, b), Coveney (2001), Feinberg and Laurentie (2006) and Hanuš *et al.* (2007). Further, there is necessary to bring out that all used indirect SCC determination methods in this paper were primarily adjusted for cow milk analyses.

Also samples of all milk kinds were analysed on SCC using fluorescent method (DCC; fluorescent signal measurement after DNA staining by propidium iodine) according to producer instruction manual (De Laval, 2006; Sweden). The relevant methodical information about method principle capability are included in other professional papers (Chemometec, 2004; Denmark). Relationships of instrumental SCC determination in milk to direct microscopic method or reference values from proficiency testing (for instance CECALAIT) were demonstrated. The linear regression relationship had form for example y = 1.05 + 15.78 for CM.

The analyses were performed in accredited National Reference Laboratory for Raw Milk (according to CSN EN ISO/IEC 17025) which is situated in Research Institute for Cattle Breeding in Rapotín (n. 1340, certificate n. 040/2005) and which co-operates in network of national reference laboratories for milk under AFSSA (Agence Francaise de Sécurité Sanitaire des Aliments) Paris supervision. In the case of high SCC in HM (> 300 10^3.ml^{-1}) a bacteriological examination on occurrence of mastitis pathogens was performed simultaneously (procedures according to: Hejlíček *et al.*, 1987; Benda and Vyletělová, 1995, 1997a, b; Benda *et al.*, 1997).

Statistical evaluation

The relationships between SCC determination results by various methods were evaluated using basic methods of difference statistics (set mean (x) and its standard deviation (sd), mean difference (d) and its variability (standard deviation, dsd) and using linear and nonlinear regression (Grappin and Jeu-

I: *SCC comparison results (10^3.ml^{-1}) among direct microscopy (DM) method, fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in cow milk (I; CM)*

	Test IA; n = 10			Test IA log				Difference					
Milk	NA	NA	NA	NA	NA	NA							
Method Column	Foss 1	DCC 2	DM 3	Foss 4	DCC 5	DM 6		1–2	1–3	2–3	4–5	4–6	5–6
x	412	367	400	2.5393	2.4957	2.5265	d	45	12	–33	0.0436	0.0128	–0.0308
sd	210	176	201	0.2786	0.2681	0.2793	dsd	44	12	34	0.0297	0.0104	0.0253
xg				346	313	336	t; sig.	3.05*	3.07*	2.91*	4.41**	3.70**	3.65**
	Test IB; n = 10			**Test IB log**									
Milk Method	NA Foss	NA DCC	NA DM	NA Foss	NA DCC	NA DM		1–2	1–3	2–3	4–5	4–6	5–6
x	419	363	404	2.5408	2.4713	2.5238	d	56	15	–41	0.0695	0.0171	–0.0525
sd	215	187	213	0.2930	0.3133	0.2945	dsd	38	8	38	0.0336	0.0117	0.0390
xg				347	296	334	t; sig.	4.36**	5.10***	3.30*	6.22***	4.37**	4.04**

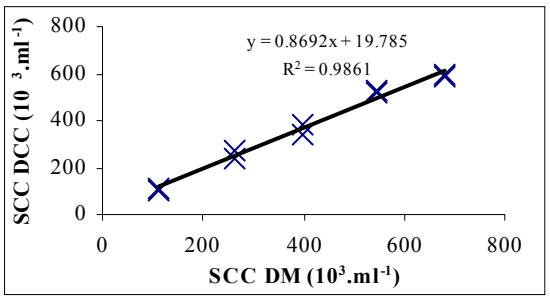
NA = native milk; FR = frozen milk; log = decadic logarithms; n = number of cases; x = arithmetic mean (10^3.ml^{-1}); sd = standard deviation for x; d = mean difference (10^3.ml^{-1}); dsd = standard deviation for d; t = pair test criterion of t–test; sig. = significance, ns = $P > 0.05$, *, **, and *** = $P \leq 0.05$, 0.01 and 0.001

net, 1974, 1975; Grappin, 1987; Hanuš *et al.*, 1993a, b, c, 2002, 2006, 2007, 2009). Linear form is hereat preferred as it is known by generally accepted calibration model for indirect methods in link to direct methods of milk analyses (Grappin, 1987; Baumgartner, 2006; Leray, 2006, 2010; Hanuš *et al.*, 2009). SCCs were evaluated in original values and also in logarithmic ($\log_{10}$) transformed form (log SCC) because of presupposed occurrence of lognormal frequency distribution in individual milk samples (Ali and Shook, 1980; Shook, 1982; Raubertas and Shook, 1982; Reneau *et al.*, 1983 and 1988; Reneau, 1986; Wiggans and Shook, 1987; Hanuš *et al.*, 1995; Janů *et al.*, 2007). Therefore also geometrical mean values (xg; mean logarithm after reverse transformation in 10^3.ml^{-1}) could be used for comparison between individual methodical SCC files. Also pair t-test was used for testing of differences between SCC means though the interpretation of these results has to be careful from analytical point of view because it can be misguided. Nevertheless, in the same time und under mentioned presupposition the pair t-test is used in result graphical interpretation of proficiency testing with indicator of Euclidian distance from origin (Leray, 1993, 2006 and 2010; Hanuš *et al.*, 1998, 2006 and 2007). In case that result repeatability of SCC measurement by various methods were calculated in data files as standard deviations it was carried out according to papers Grappin (1987) and Hanuš *et al.* (1998) by repeated SCC measurement in various milk samples. The Microsoft Excel programme was used for calculations.

RESULTS AND DISCUSSION

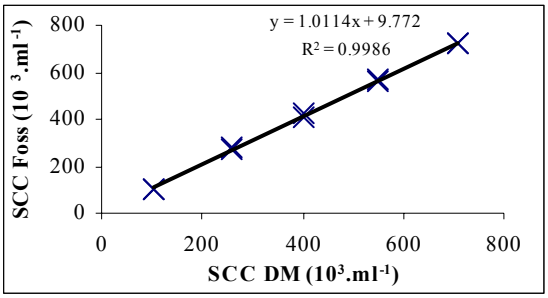
I) cow milk (CM)

Hillerton *et al.* (2004) analysed possibilities for error SCC determination in CM quality control in several studies. They mentioned that three included laboratories had differentiated samples from cows with subclinical and clinical mastitis in good way but SCC means in uninfected cows had varied between laboratories where one of laboratories had measured significantly higher. The authors recommended to eliminate these discrepancies using gliding geometric mean for SCC in the milk quality payment system which is calculated including as far as 13 values. In two tests (Tab. I) in this work very small differencies were stated in CM as compared to other kinds of milk between arithmetic and also geometric means for method (DM, Foss and DCC). In absolute values it was from 12 to 56 10^3.ml^{-1} (Tab. I). The cow milk had generally lowest values of SCC arithmetical mean using Foss method in the observed sample sets (CM < HM < SM < GM; Tab. I, VII, V and III). Mutual method result relationships between DM, Foss and DCC were significant and very tight. The correlations for DCC to DM were 0.991 and 0.993 ($P < 0.001$; Tab. II; Fig. 2), these were after log transformation more tight and similar to Foss correlations. The Foss correlations to DM were 0.989 and 0.999 ($P < 0.001$; Tab. II; Fig. 3) and after log transformation were also



Linear r = 0.993*** n = 10

2: *SCC result (10^3.ml^{-1}) relationship between direct miroscopy (DM) and DCC method in native cow milk (Tab. II), test A*
R^2 = determination coefficient; r = correlation coefficient



Linear r = 0.999*** n = 10

3: *SCC result (10^3.ml^{-1}) relationship between DM and Fossomatic (Foss) method in native cow milk (Tab. II), test B*

more tight. Also relationships between Foss and DCC were tight in CM (Tab. II; 0.989 and 0.992, $P < 0.001$). The original adjustation of all methods on cow milk showed itself fully. Most of the equations were only little different from required ideal form ($y = 1x + 0$; Grappin, 1987; Tab. II). That is reason why it is possible to consider the result correspondence or result reliability as practically very good respectively. Nevertheless, in case that DCC values should be converted to DM the following conversion linear equation $y = 1.1293x - 5.5029$ appears as suitable in CM. However, the assesment such conversion equations should be an individual matter of laboratories. In the practice and in this way all the used methods should reliably indicate a clinical but especially also subclinical mastitis in cow milk. Hering *et al.* (2008) mentioned that it was necessary to differentiate the principal and slight effects on SCC determination in correct way at result interpretation. The transport of preserved milk samples without cooling brought following results for SCC analyses in comparison to cooled samples: average differencies in SCC, fat, protein and lactose contents were irrelevant ($P > 0.05$); the straight line equations were near to ideal form (Grappin, 1987); correlation coefficients were from 0.998 and higher ($P < 0.001$) for fat and SCC; the result downgrade was not confirmed. Previously and in many countries till now preserved milk sample transport without cooling was and is used for SCC determination. As far as the time and other conditions are controlled and samples are not exposed for instance to high temperatures under the sun the mentioned fact does not have any impact on the results. Nevertheless, in the Czech Republic, there is milk sample transport for SCC determination under regime with controlled cold temperature. Hanuš *et al.* (2002 and 2008) were interested in study of temperature regime impact on SCC determination: it was demonstrated that experimental milk sample glaciation did not have an impact on SCC values; deep freeze did not have destructive effects SCC results. Benda (1995) compared the effects of chemical preservation on microbial quality of milk samples. The best preservation effect was demonstrated by bronopol and kalium bichromate. Today preservation means from D&F Control Systems, Inc., is produced on the basis of bronopol for routine sample treatment. In this way preserved milk samples did not show quality changes under laboratory temperature store for 72 hours. A comparison of preservation means regarding sample quality and SCC was published by Hanuš *et al.* (1992a, b). Also in this paper the milk sample quality changes were not documented.

II) goat milk (GM)

SCC means of A, B and C files were relatively high (Tab. III) including geometric means. However, row of papers introduced higher and high SCCs in goat milk regularly (Wilson *et al.*, 1992; Droke *et al.*, 1993; Hahn *et al.*, 1994). Sánchez–Macías *et al.* (2008) mentioned, that highest had been original SCCs and that sample temperature had lower impact on results than sample storage time for first eight hours for DCC method in goat milk. Berry and Broughan (2007) found high correlation (0.95) between SCC values from DCC and DM. Castro *et al.* (2008) noted

II: *Regression analyse results among direct microscopy (DM) method, fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in cow milk (I; CM)*

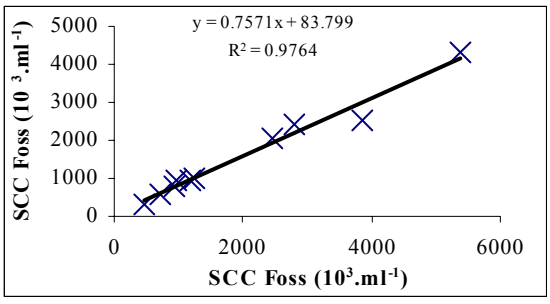
Test	x milk / method	y milk / method	Equa. type	Equation	Corr.	sig.	Note
IA n = 10	NA / DM	NA / Foss	Linear	y = 1.043x – 4.7896	0.999	***	
IA log			Linear	y = 0.9968x + 0.021	0.999	***	
IA	NA / DM	NA / DCC	Linear	y = 0.8692x + 19.785	0.993	***	Fig. 2
IA log			Linear	y = 0.9568x + 0.0783	0.997	***	
IA	NA / Foss	NA / DCC	Linear	y = 0.8294x + 25.391	0.989	***	
IA log			Linear	y = 0.9576x + 0.0641	0.995	***	
IB n = 10	NA / DM	NA / Foss	Linear	y = 1.0114x + 9.772	0.999	***	Fig. 3
IB log			Linear	y = 0.9942x + 0.0316	0.999	***	
IB	NA / DM	NA / DCC	Linear	y = 0.8692x + 11.455	0.991	***	
IB log			Linear	y = 1.0573x – 0.1969	0.994	***	
IB	NA / Foss	NA / DCC	Linear	y = 0.8597x + 2.9158	0.992	***	
IB log			Linear	y = 1.0651x – 0.235	0.996	***	

Corr. = correlation

III: *SCC comparison results (10^3.ml^{-1}) between fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in goat milk (II; GM)*

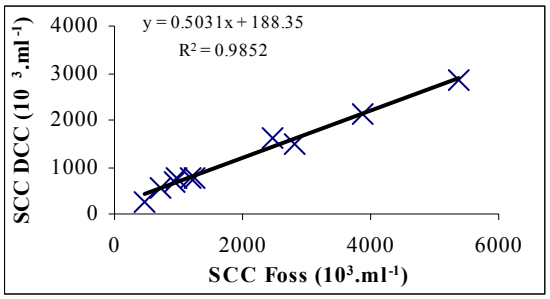
	Test IIA; n = 10			Test IIA log				Difference					
Milk	NA	FR	FR	NA	FR	FR							
Method Column	Foss 1	Foss 2	DCC 3	Foss 4	Foss 5	DCC 6		1–2	1–3	2–3	4–5	4–6	5–6
x	8677	4961	2012	3.8118	3.6323	3.2862	d	3716	6665	2949	0.1795	0.5256	0.3461
sd	7124	2740	509	0.3177	0.2323	0.1312	dsd	4528	7298	2832	0.1024	0.3640	0.2698
xg				6483	4288	1933	t; sig.	3.12*	2.46*	2.74*	5.26***	4.23***	3.85**
	Test IIB; n = 10			**Test IIB log**									
Milk Method	NA Foss	FR Foss	FR DCC	NA Foss	FR Foss	FR DCC							
x	1999	1597	1194	3.1793	3.0892	2.9847	d	402	805	403	0.0901	0.1946	0.1045
sd	1523	1167	772	0.3253	0.3201	0.2903	dsd	412	763	432	0.0414	0.0627	0.0526
xg				1511	1228	965	t; sig.	2.93*	3.17*	2.80*	6.53***	9.31***	5.96***
	Test IIC; n = 10			**Test IIC log**									
Milk Method	NA Foss	NA DCC		NA Foss	NA DCC								
x	5587	2431		3.6006	3.3268		d	3156			0.2738		
sd	4997	1300		0.3395	0.2226		dsd	3805			0.1297		
xg				3986	2122		t; sig.	2.49*			6.33***		

at SCC by DCC good correlation (0.71 and log SCC transformation decreased this correlation) to measurement results of milk colour analyse and marked it as possible good SCC predictor. However, the procedure requests more measurements. The frozen sample variants of goat milk showed good relationship between methods in this work but also to native sample variants with exception A test between Foss and DCC (Tab. IV, insignificant correlations (ns)). However, in total there was the worse agreement of mean values at SCC results of frozen samples with their native originals using Foss and DCC methods (Tab. III). In the Foss case it partly confirmed regarding relationship the previous results (Hanuš *et al.*, 2002 and 2008) in CM. In general the large differences were also between SCC geometric means and it was in all cases of tests (Tab. III). These geometric means were mutually nearest between sample variants and used methods in B test (Tab. III). This test showed also good correlation relationships between files (Tab. IV; Fig. 4, 5 and 6). However, good relationships and high correlation coefficient values in particular in native milk sample test (C; Tab. III, Tab. IV and Obr. 7 and 8) offer a possibility of measurement adjustment of SCC results by selected method ei-



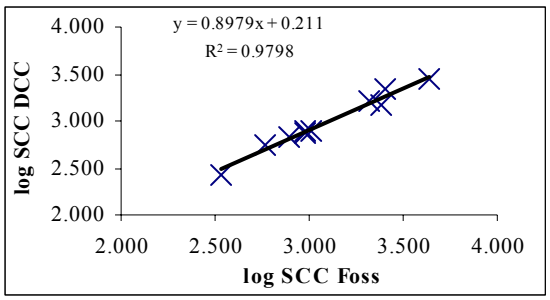
Linear r = 0.988*** n = 10

4: *SCC result (10^3.ml^{-1}) relationship between native and frozen goat milk samples using Fossomatic (Foss) method (Tab. IV), test B*



Linear r = 0.993*** n = 10

5: *SCC result (10^3.ml^{-1}) relationship between Foss and DCC method in native and frozen goat milk (Tab. IV), test B*



Linear r = 0.990*** n = 10

6: *SCC result (10^3.ml^{-1}) relationship after log transformation between Foss and DCC method in frozen goat milk (Tab. IV), test B*

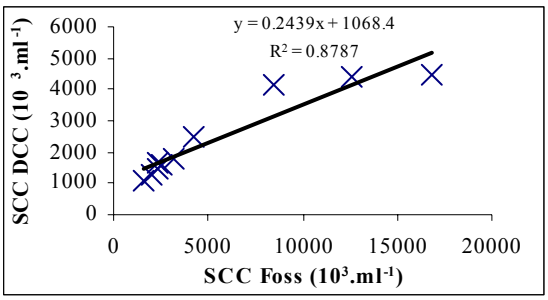
IV: *Regression analyse results between fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in goat milk (II; GM)*

Test	x milk / method	y milk / method	Equa. type	Equation	Corr.	sig.	Note
IIA n = 10	NA / Foss	FR / Foss	Linear	y = 0.372x + 1732.5	0.9672	***	
			Logarithmic	y = 3705.3Ln(x) – 27561	0.9890	***	
IIA log			Linear	y = 0.7154x + 0.9054	0.9784	***	
IIA	NA / Foss	FR / DCC	Linear	y = –0.0221x + 2204.4	–0.3100	ns	
IIA log			Linear	y = –0.0711x + 3.5573	–0.1723	ns	
IIA	FR / Foss	FR / DCC	Linear	y = –0.0167x + 2095.2	–0.0900	ns	
IIA log			Linear	y = –0.0149x + 3.3401	–0.0265	ns	
IIB n = 10	NA / Foss	FR / Foss	Linear	y = 0.7571x + 83.799	0.9881	***	Fig. 4
IIB log			Linear	y = 0.976x – 0.0137	0.9919	***	
IIB	NA / Foss	FR / DCC	Linear	y = 0.5031x + 188.35	0.9926	***	Fig. 5
IIB log			Linear	y = 0.8797x + 0.1879	0.9856	***	
IIB	FR / Foss	FR / DCC	Linear	y = 0.6502x + 155.6	0.9828	***	
IIB log			Linear	y = 0.8979x + 0.211	0.9898	***	Fig. 6
IIC n = 10	NA / Foss	NA / DCC	Linear	y = 0.2439x + 1068.4	0.9374	***	Fig. 7
			Logarithmic	y = 1640.5Ln(x) – 11170	0.9866	***	Fig. 8
IIC log			Linear	y = 0.6419x + 1.0155	0.9791	***	

ther by calibration or by recalculation for practical methodical use. Difference and correlation relationship (0.933; P < 0.001) between variants NA / Foss and FR / DCC (Tab. III, Tab. IV, test B and Fig. 5) showed that probably 98.5% of variants in FR / DCC results were explainable by variations in NA / Foss results. Similarly at correlation (0.937; P < 0.001) between variants NA / Foss and NA / DCC (Tab. IV, test C and Fig. 7) this fact showed that 87.9% of variability in DCC results was determined by variability in Foss results and the rest was affected probably by random effects. The explanation percentage of methodical dependence could be generally marked as high. In case that DCC values in GM should be converted to Foss the following conversion straight line equation could be seen as suitable: y = 3.603x – 3171.4 (processed from file C, Tab. III and IV).

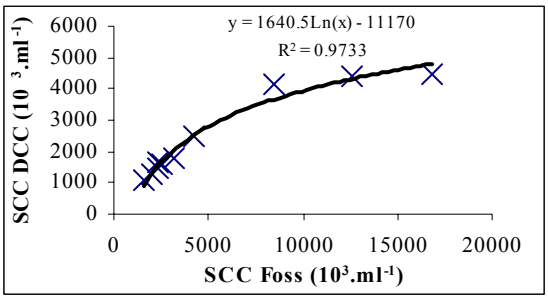
III) sheep milk (SM)

Average SCCs of files A till D (Tab. V) were middle regarding other used files and principle higher than in CM and HM but lower than in GM. Also for goat milk the literature introduced as a rule higher SCCs (Margetín *et al.*, 1995 and 1996). Frozen and native sheep milk sample variants showed good relationship (Tab. VI) and also agreement of mean values (Tab. V) especially geometric means at Foss method. In this case it confirmed well previous results (Hanuš *et al.*, 2002 and 2008) in CM. Even DCC method differences from Foss results were not too expressive. The result differences of methods (DCC – Foss) were the lowest after cow milk (CM < SM < HM < GM; Tab. I, V, VII and III). All mentioned relationships between result combinations of sample variants and used methods (Tab. VI) were tight and significant. The correlations across the tests moved from 0.920 to 0.999 (all combinations P < 0.001; Fig. 9, 10 and 11). Up to now all mentioned facts offer good possibility both for practical method use in sheep milk and for pertinent results which improve conversions. The geometric means between sample variants and used methods were mutually nearest in C test (Tab. V). Difference and correlation relationship (0.998; P < 0.001; Fig. 10) between variants FR



Linear r =0.937*** n = 10

7: *SCC result (10³.ml⁻¹) relationship between Foss and DCC method in native goat milk (Tab. IV), test C*

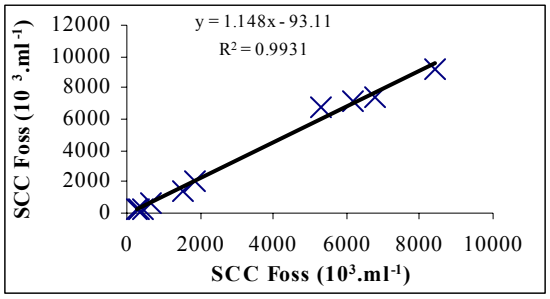


Logarithmic r = 0.987*** n = 10

8: *Log SCC result (10³.ml⁻¹) relationship between Foss and DCC method in native goat milk (Tab. IV), test C*

V: *SCC comparison results (10^3.ml^{-1}) between fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in sheep milk (III; SM)*

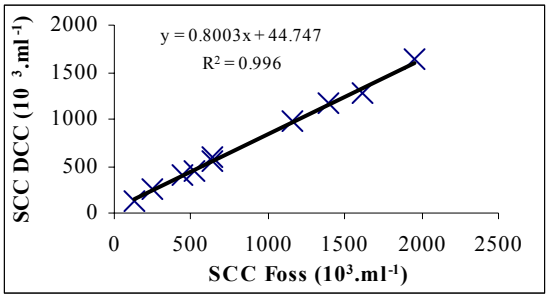
	Test IIIA; n = 10			Test IIIA log				Difference					
Milk	NA	FR	FR	NA	FR	FR							
Method **Column**	Foss 1	Foss 2	DCC 3	Foss 4	Foss 5	DCC 6		1–2	1–3	2–3	4–5	4–6	5–6
x	3168	3544	1667	3.2116	3.2151	3.0602	d	–376	1501	1876	–0.0035	0.1515	0.1549
sd	2978	3431	1101	0.5337	0.6064	0.4331	dsd	525	2013	2453	0.0746	0.1984	0.2416
xg				1628	1641	1149	t; sig.	2.15^{ns}	2.24^{ns}	2.30^{ns}	0.14^{ns}	2.29^{ns}	1.92^{ns}
	Test IIIB; n = 10			Test IIIB log									
Milk **Method**	NA Foss	FR Foss	FR DCC	NA Foss	FR Foss	FR DCC							
x	726	875	745	2.7685	2.8174	2.7665	d	–149	–19	130	–0.0489	0.0020	0.0509
sd	417	587	470	0.3108	0.3590	0.3260	dsd	179	69	121	0.0610	0.0339	0.0372
xg				587	657	584	t; sig.	2.49*	0.82^{ns}	3.22*	2.40*	0.18^{ns}	4.10**
	Test IIIC; n = 10			Test IIIC log									
Milk **Method**	NA Foss	FR Foss	FR DCC	NA Foss	FR Foss	FR DCC							
x	486	441	401	2.5945	2.5610	2.5361	d	45	85	40	0.0335	0.0584	0.0249
sd	259	233	191	0.3291	0.2980	0.2650	dsd	38	74	48	0.0618	0.0849	0.0381
xg				393	364	344	t; sig.	3.58**	3.47**	2.49*	1.63^{ns}	2.06^{ns}	1.96^{ns}
	Test IIID; n = 10			Test IIID log									
Milk **Method**	NA Foss	NA DCC		NA Foss	NA DCC								
x	611	456		2.6967	2.5654		d	155			0.1313		
sd	427	306		0.2681	0.2844		dsd	132			0.0731		
xg				497	368		t; sig.	3.52**			5.39***		



Linear r = 0.997*** n = 10

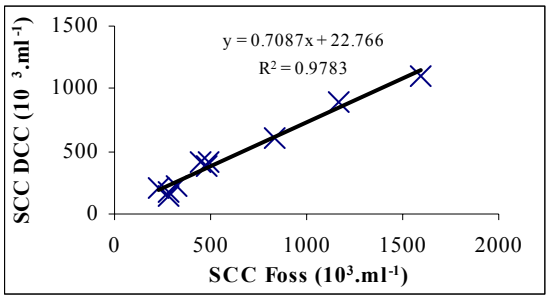
9: *SCC result (10^3.ml^{-1}) relationship between native and frozen sheep milk using Foss method (Tab. VI), test A*

/ Foss and FR / DCC (Tab. V, Tab. VI, test B) showed that probably 99.6% of variations in FR / DCC results were explainable by variations in FR / Foss results. By analogy at correlation (0.989; P < 0.001) between variants NA / Foss and NA / DCC (Tab. VI, test D and Fig. 11) it was shown that 97.8% of variability in DCC results was caused by Foss result variability and the rest was influenced probably by random effects. The explanation percentage of methodical dependence could be generally marked as high. This was also higher as compared to GM files. However, for conversion assesment the method results from native milk were preferred because of methodical correct-



Linear r = 0.998*** n = 10

10: *SCC result (10^3.ml^{-1}) relationship between Foss and DCC method in frozen sheep milk (Tab. VI), test B*



Linear r = 0.989*** n = 10

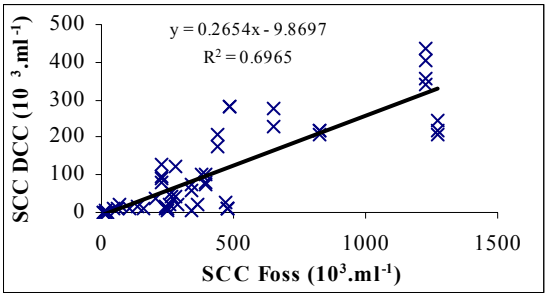
11: *SCC result (10^3.ml^{-1}) relationship between Foss and DCC method in native sheep milk (Tab. VI), test D*

VI: *Regression analyse results between fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in sheep milk (III; SM)*

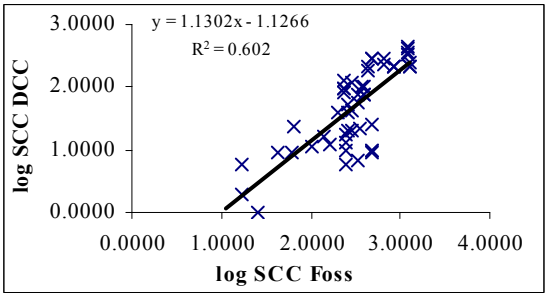
Test	x milk / method	y milk / method	Equa. type	Equation	Corr.	sig.	Note
IIIA n = 10	NA / Foss	FR / Foss	Linear	y = 1.148x – 93.11	0.9965	***	Fig. 9
IIIA log			Linear	y = 1.0906x – 0.2873	0.9958	***	
IIIA	NA / Foss	FR / DCC	Linear	y = 0.34x + 590.25	0.9196	***	
			Logarithmic	y = 851.36Ln(x) – 4628.4	0.9857	***	
IIIA log			Linear	y = 0.7418x + 0.6779	0.9483	***	
IIIA	FR / Foss	FR / DCC	Linear	y = 0.296x + 618.42	0.9222	***	
			Logarithmic	y = 777.77Ln(x) – 4090.5	0.9861	***	
IIIA log			Linear	y = 0.6757x + 0.8876	0.9460	***	
IIIB n = 10	NA / Foss	FR / Foss	Linear	y = 1.3979x – 140.01	0.9934	***	
IIIB log			Linear	y = 1.1482x – 0.3614	0.9938	***	
IIIB	NA / Foss	FR / DCC	Linear	y = 1.1229x – 70.29	0.9950	***	
IIIB log			Linear	y = 1.0444x – 0.1248	0.9955	***	
IIIB	FR / Foss	FR / DCC	Linear	y = 0.8003x + 44.747	0.9980	***	Fig. 10
IIIB log			Linear	y = 0.9069x + 0.2115	0.9987	***	
IIIC n = 10	NA / Foss	FR / Foss	Linear	y = 0.8957x + 5.0406	0.9932	***	
IIIC log			Linear	y = 0.8923x + 0.2459	0.9854	***	
IIIC	NA / Foss	FR / DCC	Linear	y = 0.7321x + 44.68	0.9907	***	
IIIC log			Linear	y = 0.7908x + 0.4845	0.9822	***	
IIIC	FR / Foss	FR / DCC	Linear	y = 0.8145x + 41.836	0.9939	***	
IIIC log			Linear	y = 0.887x + 0.2644	0.9977	***	
IIID n = 10	NA / Foss	NA / DCC	Linear	y = 0.7087x + 22.766	0.9891	***	Fig. 11
IIID log			Linear	y = 1.0256x – 0.2002	0.9667	***	

ness. In case that DCC values in SM should be converted to Foss the following conversion straight line equation could be seen as suitable:

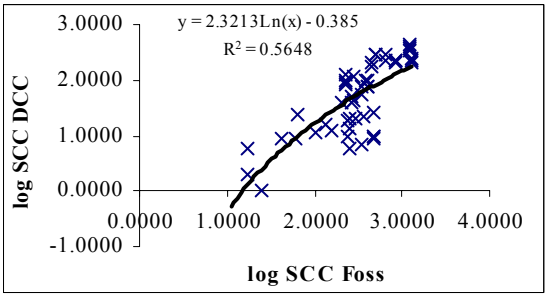
y = 1.3805x – 18.149 (processed from file D, Tab. V and VI).



Linear r = 0.835*** n = 54



Linear r = 0.776*** n = 52



Logarithmic r = 0.752*** n = 52

12: *SCC result (10^3.ml^{-1}) relationship between Fossomatic and DCC method in human milk using regression method*

VII: *SCC comparison (10^3.ml^{-1}) and regression analyse results between fluoro–opto–electronic (Fossomatic; Foss) and fluorescent (De Laval; DCC) method in human milk (IV; HM)*

	Test IVA; n = 54		Test IVA log			Difference	
Milk	FR	FR	FR	FR			
Method	Foss	DCC	Foss	DCC		1–2	3–4
Column	1	2	3	4			
x	437	106	2.4336	1.6845	d	331	0.8115
sd	369	117	0.5147	0.6476	dsd	278	0.4074
xg			271	48	t; sig.	8.66***	14.50***

Test	x milk / method	y milk / method	Equa. type	Equation	Corr.	sig.	Note
IVA n=54	FR / Foss	FR / DCC	Linear	y = 0.2654x – 9.8697	0.835	***	Fig. 12
IVA log				y = 1.1302x – 1.1266	0.776	***	Fig. 12
			Logarithmic	y = 2.3213Ln(x) – 0.385	0.752	***	Fig. 12

IV) human milk (HM)

Human milk is not tested very often both for composition and from analytical and methodical point of view. This is valid also for medicine literature. However, recently some tests were carried out as comparison to cow, goat and sheep milk (Hanuš *et al.*, 2009 a 2010). The existence of relatively good correlation relationship between SCC (r = 0.84; P < 0.001; Fig. 12) obtained by both methods (Foss and DCC) and hereat marked difference between SCC means (Tab. VII; geometric means 271 and 48 10^3.ml^{-1}, P < 0.001) could be explained either by difference in cell nucleus size in different biological kind of milk thereby by smaller quantity of their emission in HM or by less intesive binding dyes to nucleus DNA ibidem or by longer penetration of dye through membranes into cells in HM respectively at DCC method. Furthermore it means that 70.6% of SCC variations according to DCC method are explainable by SCC variability according to Foss method. Certain portion remains so on random variations which are caused by various interference effects. In principle the DCC method is applicable also in human milk. Here is possible to perform a correction recomputation according to relevant transformation equation. It is possible to reason about using such equation hence that paper by Hanuš *et al.* (2009) documented good Foss SCC result correspondence with examination using direct microscopic method in this concrete case. The DCC method could be usable to somatic cell quantity in HM after conversion of DCC result to real values. Considering mentioned correlation relationship it is possible to expect an exploitable relationship also in the DCC case to direct microscopic method as reference and derive such conversion relation. In case that DCC values in HM should be converted to Foss (real SCC respectively) the following conversion straight line equation could be seen as suitable: y = 2.6246x + 158.63. Measurement repeatability evaluation at DCC method showed on value ± 29 10^3.ml^{-1} (it means. ± 17.1%) in frozen human milk (n = 17; x = 170 10^3.ml^{-1}) in the range from 2 to 438 10^3.ml^{-1} (from 7 to 1273 10^3.ml^{-1} for Foss).

According to previous results (Hanuš *et al.*, 2009) this is possible to carry out SCC analyses using Fossomatic 90 (fluoro-opto-elektronic counting on rotation disc) with acceptable result reliability (as compared to direct microscopic method) in more biological kinds of milk (mammal species, such as cow, sheep, goat and human milk) with calibration for instance on cow milk, it means without specific species calibrations. Nevertheless, according results by Zeng (1996) and Zeng *et al.* (1999) there were found higher goat SCCs by 27.0 and 24.6% in case of Fossomatic calibration by cow milk sample standards for SCC, which could indicate a need such specific calibrations. Also at instrumental (Bacto-Scan, Foss Electric) assesment of total bacteria count in GM (Tomáška *et al.*, 2006) the specific species calibrations (equations) were shown as compared to CM as necessary. However, in this work we proceeded according to our previous results which warranted this above mentioned procedure. From interpretation point of view it is interesting without question that all carried out bacteriological examinations because of mastitis pathogens were negative at higher SCCs in HM. Therefore, it was probably not an infectious mastitis but rather a nonspecific state of mild milk secretion disorders.

CONCLUSION

The results showed relatively good capability and correspondence of methods (direct optical microscopy, fluoro-opto-electronic (Fossomatic) and DCC) at SCC measurement and reliability of reached results in cow, goat, sheep and human milk for milk quality and mammary gland health control. The results made possible to obtain a near image about correspondence (difference variability respectively) of SCC results which were stated using various methods in various biological kinds of milk. DCC method gave lower results in small ruminant milk as compared to Fossomatic method calibrated on cow milk by dirrect microscopic method. In human milk the DCC method gave markedly lower results as compared to Fossomatic method adjusted to cow milk at good correlation, which is possible to solve

by stated transformation equation. The following suitable conversion equations were calculated for recomputation from DCC results: to DM in cow milk y = 1.1293x – 5.5029; to Foss in goat milk y = 3.603x – 3171.4; to Foss in sheep milk y = 1.3805x – 18.149; to Foss in human milk y = 2.6246x + 158.63. However, the assesment of such conversion equations should be individual matter. Nevertheless, in general in all cases of using of conversion equations the specific calibrations would be more advantageous. The methodical results of paper are usable in routine practice of milk laboratories at milk quality control and mammary gland health control during lactation of mammals females.

SUMMARY

Somatic cell count (SCC) is important foodstuff and hygienic (bulk samples) and also health indicator (individual samples) of animal mammary gland. SCC is a number of leucocytes in milk as nucleated cells. These represent an actual state of physiological and pathological activity of animal defensive system regarding possible mammary gland infection. SCC values show on occurrence frequency of milk secretion disorders. SCC determination serves to control of milk food chain quality at its beginning. Milk market is extending by products prepared from various kinds (species) of alternative milk as compared to cow milk. SCC determination in other various milk kinds of other farm animals assumes importance all the time. The goal of this paper was to evaluate the ability of chosen analytical procedures to production fo SCC reliable results in various milk types from biological species. The various methods of SCC determination were compared by model sets of cow (CM), goat (GM), sheep (SM) and human (HM) milk: direct optical microscopy (DM; CSN EN ISO 13366–1); fluoro-opto-electronic microscopy on disc rotation (Fossomatic 90; Foss; CSN EN ISO 13366–2); fluorescent method (DCC; De Laval). The result correspondence and differences were assessed by basic statistical procedures including linear regression model. Used indirect instrumental methods used cow milk calibration basically. The DM, Foss and DCC mutual result relations about SCC were only with small exception very close for CM, GM, SM and HM. It was mostly $\geq$ 0.92 ($P < 0.001$) for CM, GM and SM. In particular in CM the regression equations between methods were near ideal form y = 1x + 0. Goat milk had the highest mean SCC values (GM > SM > HM > CM) in tested files. The mean differences SCC data sets between mentioned methods were small for cow milk, larger for sheep milk and human milk and the largest for goat milk. The result differences of method DCC – Foss were in following order CM < SM < HM < GM. Despite this fact it is possible to convert all DCC results in sheep, human and goat milk to DM or Foss comparative method. The following suitable conversion equations were stated for calculation from DCC: to DM in cow milk y = 1.1293x – 5.5029; to Foss in goat milk y = 3.603x – 3171.4; to Foss in sheep milk y = 1.3805x – 18.149; to Foss in human milk y = 2.6246x + 158.63. However, assesment of such conversion equations should be individual laboratory event. The results showed relatively good method correspondence (among DM, Foss and DCC) for SCC determination and reliability of achieved results in cow, goat, sheep and human milk for quality and mammary gland health state control. DCC method offered lower results in small ruminants milk as compared to Foss method calibrated on cow milk using DM. DCC method in human milk offered markedly lower results as compared to Foss method adjusted by cow milk at good correlation (0.84; $P < 0.001$) which is possible to solve using stated transformation equation. However, in general in all cases of using of conversion equations the specific calibrations would be more advantageous. Methodical results of work are usable in routine practice of milk laboratories in milk quality control and in control of mammary gland health in lactation of mammals females.

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