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THE EVALUATION OF REAL TIME MILK ANALYSE RESULT RELIABILITY IN THE CZECH REPUBLIC

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Abstract

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The good result reliability of regular analyzes of milk composition could improve the health monitoring of dairy cows and herd management. The aim of this study was the analysis of measurement of abilities and properties of RT (Real Time) system (AfiLab = AfiMilk (NIR measurement unit (near infrared spectroscopy) and electrical conductivity (C) of milk by conductometry) + AfiFarm (calibration and interpretation software)) for the analysis of individual milk samples (IMSs). There were 2 × 30 IMSs in the experiment. The reference values (RVs) of milk components and properties (fat (F), proteins (P), lactose (L), C and the somatic cell count (SCC)) were determined by conventional (direct and indirect: conductometry (C); infrared spectroscopy 1) with the filter technology and 2) with the Fourier transformations (F, P, L); fluoro-opto-electronic cell counting (SCC) in the film on the rotation disc (1) and by flow cytometry (2)) methods. AfiLab method (alternative) showed less close relationships as compared to the RVs as relationships between reference methods. This was expected. However, these relationships (r) were mostly significant: F from .597 to .738 ($P \leq 0.01$ and ≤ 0.001); P from .284 to .787 ($P > 0.05$ and $P \leq 0.001$); C .773 ($P \leq 0.001$). Correlations (r) were not significant ($P > 0.05$): L from –.013 to .194; SCC from –.148 to –.133. Variability of the RVs explained the following percentages of variability in AfiLab results: F to 54.4 %; P to 61.9 %; L only 3.8 %; C to 59.7 %. Explanatory power (reliability) of AfiLab results to the animal is increasing with the regularity of their measurements (principle of real time application). Correlation values r (x minus 1.64 × sd for confidence interval (one-sided) at a level of 95 %) can be used for an alternative method in assessing the calibration quality. These limits are F 0.564, P 0.784 and C 0.715 and can be essential with the further implementation of this advanced technology of dairy herd management.

Keywords: cow, raw milk, flow near infrared spectroscopy, result reliability, fat, protein, lactose, electrical conductivity, somatic cell count

INTRODUCTION

Recently, significant progress was made in the field of automation technology the physical and chemical analysis of various biological materials. These include solutions such as body fluids including milk (Katz, 2007; Karp and Petersson Wolfe, 2010; Durkin *et al.*, 2012). Also real time (RT) analyses are output as a result of the development of software for the aforementioned analytical processes. These procedures are implemented as an automated version of the milking parlours (Katz, 2007; Katz and

Pinsky, 2008; Kamphuis *et al.*, 2008 a, b, Kawasaki *et al.*, 2008, 2010; Diepersloot, 2011; Ishay *et al.*, 2011) and robotic milking. The measuring units are built into the flow system. The farmer receives information on milk quality and health of dairy cows regularly at each milking.

Such milking systems generally measure the time of milking, milk yield (kg), the milk temperature, the conductivity (C), then the fat (F) protein (P) and lactose (L) content and the somatic cell count (SCC). This creates a virtually continuous use database information. This is suitable for the management of dairy herds. For instance cow nutrition and prevention and treatment of production disorders can be corrected in this way. The combination of the values as F, P, L and milk yield in early lactation (F/P and F/L) allows control of subclinical ketosis occurrence (Geishauser and Ziebell, 1995, Duffield *et al.*, 1997, 2009; Reist *et al.*, 2002; Knegsl *et al.*, 2010; Hanuš *et al.*, 2011 c; Durkin *et al.*, 2012; van der Drift *et al.*, 2012; Manzenreiter *et al.*, 2013). Furthermore, the combined values of C, L, SCC and milk yield (L/log SCC, L/C) allows control of subclinical and clinical mastitis occurrence (Hanuš *et al.*, 1992; Pyorälä, 2003; Lukas *et al.*, 2005; Katz, 2007; Park *et al.*, 2007; Karp and Petersson Wolfe, 2010; Ishay *et al.*, 2011; Petersson Wolfe, 2013). The aim of these interpretive methods is to improve cow health, reproduction and longevity, quality of milk and then breed economy. Therefore, it is important the accuracy of the results of RT milk analyzes.

Important for the result reliability of indirect methods are the results of the reference methods (Grappin, 1987, 1993) used to calibrate them (Fig. 1, alpha level). Another important factor on the field of dairy analyses is the formation of laboratory network and proficiency testing (PT) organization (Vines *et al.*, 1986; Arndt *et al.*, 1991; Leray, 1993, 2007, 2009, Heeschen *et al.*, 1994; Wood, 1994; Golc Teger *et al.*, 1996; Golc Teger, 1997; Fuchs, 2000; Barbano, 2009; Baumgartner, 2009; Castaneda, 2009; Hanuš *et al.*, 2011 a).

Used indirect measurement procedures are in milk composition (major components) usually physical methods. In addition to the MIR and MIR–FT (mid infrared; Leray, 1993, 2007; Baumgartner, 2009; Barbano, 2009; Castaneda, 2009; Knegsel *et al.*, 2001; Hanuš *et al.*, 2011 a; van der Drift *et al.*, 2012) are also usually the NIR method (near infrared; Tsenkova *et al.*, 1999 and 2000; Kukačková *et al.*, 2000; Jankovská and Šustová, 2003; Šustová *et al.*, 2007), nephelometry, colorimetry and ultrasonic method. This last is working as analysis of response and modification of ultrasound by organic materials. In terms of flow analysis (RT) during milking there are difficult measurement conditions such as flow, foaming and lack of time when unstable material environment. Meanwhile, there is probably only suitable method of analysis of optical radiation (NIR; Katz, 2007; Katz and Pinsky, 2008; Karp and Petersson Wolfe, 2010; Ishay *et al.*, 2011) with a laser source (AfiLab) after passing through the milk. Two application problems are arising from mentioned conditions:

1. verification and validation of the measurement system with regard to calibration, reliability, accuracy, repeatability, reproducibility and competence in the proficiency testing of analytical work, or estimate the uncertainty of measurement results – application of the measuring system;
2. procedures for effective interpretation of test results (database) in the management of dairy herds – application of measurement results.

The hypothesis is the assumption that real time (RT) analysis of milk is equivalent to conventional laboratory procedures regarding the result reliability. The aim of this study was to analyze the measuring options and features of RT system (AfiLab = AfiMilk (NIR measurement unit (near infrared spectroscopy) and electrical conductivity (C) of milk by conductometry) + AfiFarm (calibration and interpretation software)) for the analysis of individual milk samples in the Czech Republic. Good reliability of the results could improve the health monitoring and control of dairy cows.

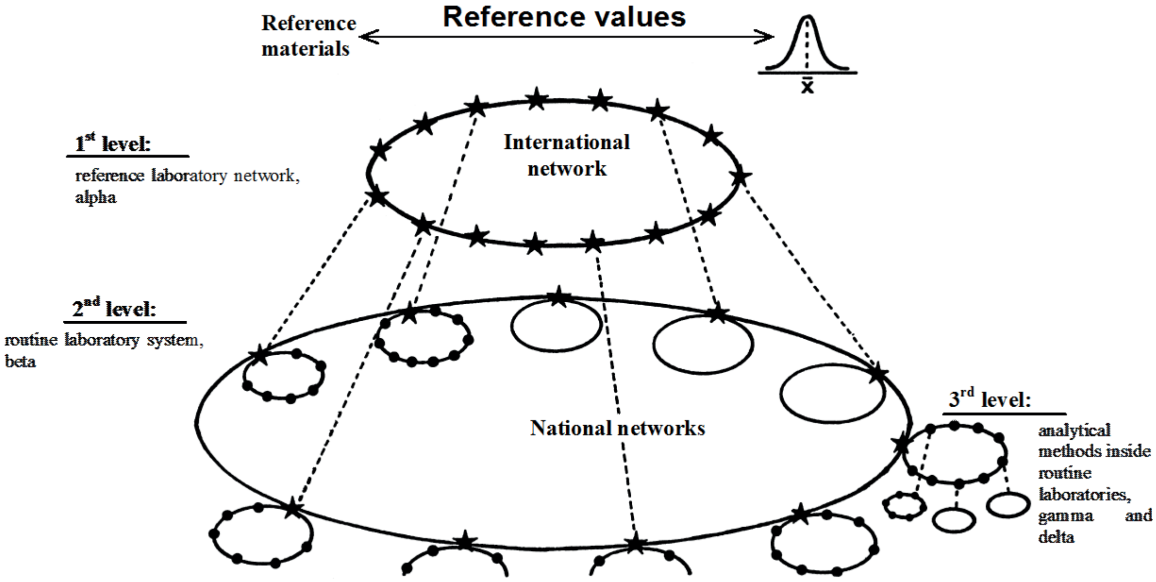
MATERIALS AND METHODS

Experiment locality, animals and milk samples

Two comparative experiments (n = 2 × 30 milk samples) were conducted in two sampling dates on agriculture farm (50°2'33.815"N, 16°26'54.534"E; altitude 400 m). In the morning milking (first of three a day) at randomly selected six positions of the measuring units AfiLab in parlour (side by side, 2 × 14, Fullwood) 30 individual milk samples (2 ×) were collected. 30 Holstein cows on different lactations (40 % of the first lactation) and at different stages of lactation were selected randomly. Composition of individual milk samples was measured and recorded via AfiLab. Further, the samples were cooled and transported to the laboratory measurements by reference methods.

Analytic procedures

The first installation of milking parlours with the RT milk analysis (AfiLab) were made in the Czech Republic. To assess the reliability of the results it was necessary to perform the experimental comparison of the results of relevant analytical methods. In this AfiLab case (Katz, 2007; Karp and Petersson Wolfe, 2010; Ishay *et al.*, 2011) a transfer of calibration from routine (indirect) methods of milk recording laboratories is performed (from level beta to gamma or delta, Fig. 1). This procedure is specific for the environment of milking parlours. In the laboratory environment (Grappin, 1987, 1993, Leray, 1993, 2007, 2009; Barbano, 2009; Baumgartner, 2009; Castaneda, 2009; Hanuš *et al.*, 2011 a) it is used only rarely (mostly from alpha to beta, Fig. 1).



1: *Scheme of reference-routine network of milk laboratories from viewpoint of efficiency of quality assurance system (modified according to Grappin, 1993)*

Reference methods for reference instruments were: for F the extraction and gravimetric method according to Röse Gottlieb in %; for L the polarimetric method in % of monohydrate; for P the method according to Kjeldahl by mineralization, steam distillation and titration, total N × 6.38 in % of crude protein; for SCC direct microscopy after staining of cells, 10^3.ml^{-1}; for C the conductometry using apparatus Radelkis OK 102/1 with bell glass electrode (Radelkis, Hungary) in mS.cm^{-1} (at 20 °C with calibration by KCL solution 10.2 mS.cm^{-1}).

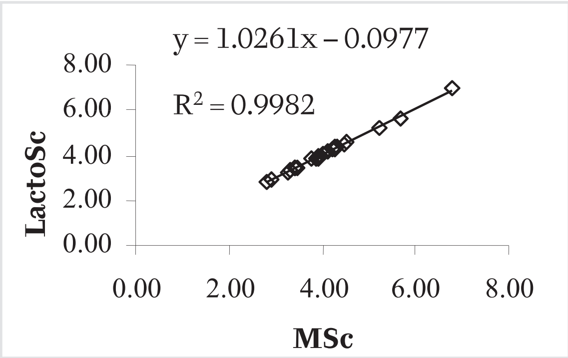
F, P, L, SCC and C were determined by various indirect methods: 1) F, P and L using MIR (infrared (IR) technology with optical filters), 1 MilkoScan 133B (Foss Electric, Denmark), the reference values; 2) F, P and L, using MIR–FT (IR spectroscopy of whole spectrum by Michelson's interferometer and using Fourier's transformations), 1 Lactoscope FTIR (Delta Instruments, The Netherlands) and 1 CombiFoss MilkoScan FT 6000 (Foss Electric, Denmark), the reference values; 3) SCC fluoro-opto-electronically (this method is comparable to direct method of SCC determination) using Fossomatic (DC, counting technology of stained cells in the endless film band on a rotation disc), 1 Fossomatic 90 (Foss Electric, Denmark) and SCC fluoro–opto–electronically using flow cytometry (FC, this method is also comparable to direct method of SCC determination, counting technology of stained cells in the stream of buffer solution), 1 CombiFoss Fossomatic FC (Foss Electric, Denmark), the reference values; 4) C using potentiometric conductometry, 1 Radelkis OK 102/1, the reference values; 5) F, P, L, SCC and C using AfiLab (in-line, free-flow and non-interfering measuring by near infra-red spectroscopy principle (Tsenkova *et al.*, 1999 and 2000; Katz, 2007; Katz and Pinsky, 2008) including potentiometric measuring of milk electrical conductivity (C)), dependent values.

The analytical methods, calibration and operation of instruments were carried out in accordance with relevant standards (CSN 57 0530, 57 0536, CSN EN ISO 17025, 13366-1 (0531 57) 13366-2 (57 0531)) and the manufacturer's manuals.

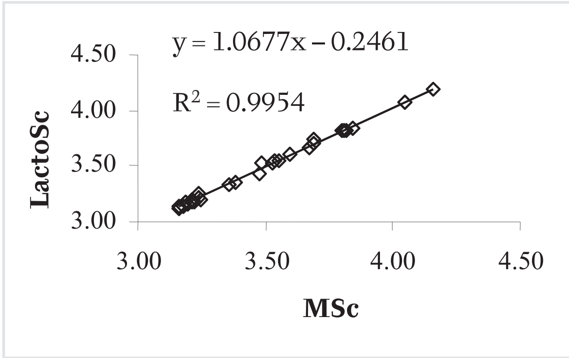
Statistic evaluation

The basic statistical characteristics were calculated for individual data files (n = 30 measurements in the set) using MS Excel (Microsoft, Redmond, USA). Also difference statistic and linear regression was performed. Important thing is the closeness of result relationships of compared reference methods and AfiLab results. Shift of regression line on the axis can be easily corrected. Therefore, following parameters were included in evaluation: determination coefficient (R^2); correlation coefficient (r); standard deviation of the mean of individual differences (sd). Mentioned parameters are essentially independent on the shift of regression line and in practice relatively little affected by relevant slope.

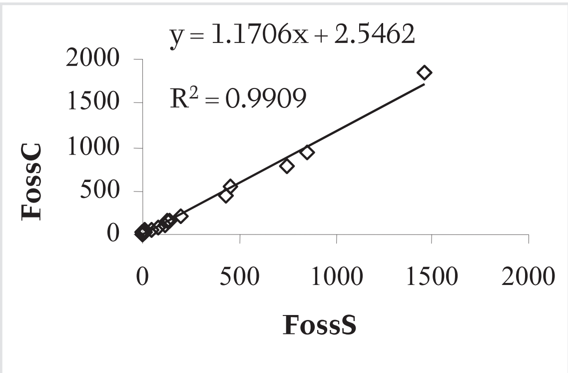
SCC values were transformed on $\log_{10}$ basis because of absence of normal frequency data distribution in SCC of individual milk samples. According to the specifications of AfiLab, the SCC was expressed semi-quantitatively in three classes (Katz, 2007; Katz and Pinsky, 2008): < 200; 200–400; 401–800; > 800 10^3.ml^{-1}. Therefore, for the purposes of statistic result reliability evaluation (comparison to reference values), that this was feasible, mentioned classes were transformed into wildcard SCC values: 100; 300; 600; 1200 10^3.ml^{-1}.



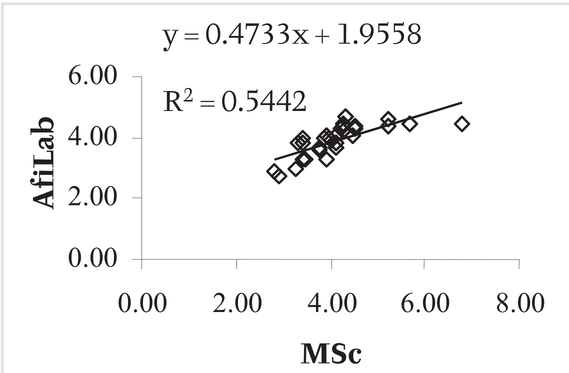
2: *Linear regression of relationship of indirect methods for reference results of milk fat (%)*
n = 30; r = 0.999 (P ≤ 0.001)



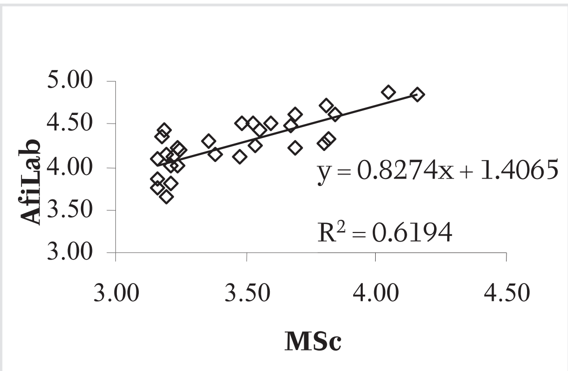
3: *Linear regression of relationship of indirect methods for reference results of milk protein (%)*
n = 30; r = 0.998 (P ≤ 0.001)



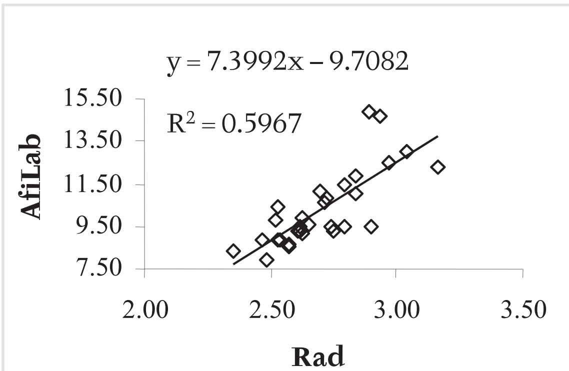
4: *Linear regression of relationship of methods for reference results of somatic cell count in milk (10^3.ml^{-1})*
n = 30; r = 0.995 (P ≤ 0.001)



5: *Linear regression of relationship between indirect method for reference results (MSc) and AfiLab equipment (alternative method) for milk fat (%)*
n = 30; r = 0.738 (P ≤ 0.001)



6: *Linear regression of relationship between indirect method for reference results (MSc) and AfiLab equipment (alternative method) for milk protein (%)*
n = 30; r = 0.787 (P ≤ 0.001)



7: *Linear regression of relationship between results of reference method (Rad; mS.cm^{-1}) and AfiLab equipment (alternative method) in milk electrical conductivity*
n = 30; r = 0.773 (P ≤ 0.001)

I: *Basic statistical parameters of milk indicators of various analytical methods, first experiment*

C/P	F	F	F	P	P	P	L	L	L	C	C	SCC	SCC	log SCC	log SCC
met	MIR	MIR-FT	NIR	MIR	MIR-FT	NIR	MIR	MIR-FT	NIR	Pot	Pot	DC	NIR+Pot	DC	NIR+Pot
app	MSc	LactoSc	AfiLab	MSc	LactoSc	AfiLab	MSc	LactoSc	AfiLab	Rad	AfiLab	FossS	AfiLab	FossS	AfiLab
unit	%	%	%	%	%	%	%	%	%	$mS.cm^{-1}$	-	$10^3.ml^{-1}$	$10^3.ml^{-1}$	-	-
n	30	30	30	30	30	30	30	30	30	30	30	30	25	30	25
x	4.12	4.13	3.9	3.47	3.46	4.28	4.75	4.78	3.65	2.7	10.29	182	964	1.8997	2.8704
xg														79	742
sd	.817	.839	.524	.29	.31	.305	.258	.232	.697	.186	1.78	331	438	.523	.409
v %	19.8	20.3	13.4	8.4	9.0	7.1	5.4	4.9	19.1	6.9	17.3	182	45.5	-	-
min	2.83	2.84	2.71	3.16	3.12	3.65	4.12	4.26	2.69	2.35	7.9	11	100	1.0414	2
max	6.79	6.97	4.68	4.16	4.18	4.88	5.15	5.13	5.18	3.16	14.9	1,720	1,200	3.2355	3.0792
m	4.07	4.07	4.0	3.43	3.39	4.25	4.81	4.87	3.59	2.67	9.55	70	1,200	1.8414	3.0792

C/P–component/property; met–method; app–apparatus; F–fat content; P–crude protein content; L–lactose monohydrate content; C–electrical conductivity; SCC–somatic cell count; log–decadic logarithm; MIR–mid infrared spectroscopy; MIR-FT–MIR with Fouriers' transformation; NIR–near infrared spectroscopy; Pot–potentiometric conductivity measuring; DC–rotation disc cytometry; FC–flow cytometry; MSc–MilkoScan 133 B; LactoSc–Lactoscope FTIR; FossC–CombiFoss MilkoScan FT 6000 and Fossomatic FC; Rad–Radelkis OK 102/1; FossS–Fossomatic 90; n–number of cases; x– arithmetic mean; xg–geometric mean; sd–standard deviation; v–variation coefficient; min–minimum; max–maximum; m–median.

II: *Basic statistical parameters of milk indicators of various analytical methods, second experiment*

C/P	**F**	**F**	**F**	**P**	**P**	**P**	**L**	**L**	**L**	**SCC**	**SCC**	**SCC**	**log SCC**	**log SCC**	**log SCC**
met	MIR	MIR-FT	NIR	MIR	MIR-FT	NIR	MIR	MIR-FT	NIR	DC	FC	NIR+Pot	DC	FC	NIR+Pot
app	MSc	FossC	AfiLab	MSc	FossC	AfiLab	MSc	FossC	AfiLab	FossS	FossC	AfiLab	FossS	FossC	AfiLab
unit	%	%	%	%	%	%	%	%	%	$10^3.ml^{-1}$	$10^3.ml^{-1}$	$10^3.ml^{-1}$	-	-	-
n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
x	3.45	3.41	3.64	3.06	3.09	4.28	4.94	4.87	2.92	162	192	430	1.3567	1.7296	2.3238
xg													23	54	211
sd	.641	.677	.58	.367	.333	.232	.144	.146	.346	330	388	513	.907	.653	.503
v %	18.6	19.9	15.9	12	10.8	5.4	2.9	3	11.8	204	202	119	67	-	-
min	2.49	2.38	2.65	2.66	2.69	3.76	4.49	4.41	2.36	2	9	100	.301	.9542	2
max	5.88	5.98	4.99	4.29	4.2	4.82	5.19	5.11	4.1	1,467	1,839	1,200	3.1664	3.2646	3.0792
m	3.36	3.33	3.53	2.96	2.99	4.27	4.93	4.87	2.97	8	33	100	2.1047	2.1375	3.0792

III: *Determination (R^2; %) and correlation (r) coefficients of analytical results from linear regression in milk components and properties between methods and instruments, first experiment*

C/P	**met**	**app**	**F**	**F**	**P**	**P**	**L**	**L**	**C**	**SCC**	**log SCC**
R^2/r											
met			MIR-FT	NIR	MIR-FT	NIR	MIR	MIR-FT	Pot	DC	DC
app			LactoSc	AfiLab	LactoSc	AfiLab	MSc	LactoSc	AfiLab	FossS	FossS
F	MIR	MSc	**99.8/.999**	**54.4/.738**							
F	MIR-FT	LactoSc		**53.8/.733**							
P	MIR	MSc			**99.5/.998**	**61.9/.787**					
P	MIR-FT	LactoSc				**61.8/.786**					
L	MIR	MSc						**98.8/.994**			
L	NIR	AfiLab					*0.02/-.013*	*0/-.001*			
C	Pot	Rad							**59.7/.773**		
SCC	NIR+Pot	AfiLab								*1.8/-.133*	
log SCC	NIR+Pot	AfiLab									*1.8/-.133*

Statistical significance: no significant $P > 0.05$ is italics letter; significant $P < 0.01$ is normal letter; significant $P < 0.001$ is boldface.

IV: *Determination (R^2; %) and correlation (r) coefficients of analytical results from linear regression in milk components and properties between methods and instruments, second experiment.*

C/P	**met**	**app**	**F**	**F**	**P**	**P**	**L**	**L**	**SCC**	**SCC**	**log SCC**	**log SCC**
R^2/r												
met			MIR-FT	NIR	MIR	MIR-FT	MIR	MIR-FT	DC	FC	DC	FC
app			FossC	AfiLab	MSc	FossC	MSc	FossC	FoosS	FossC	FoosS	FossC
F	MIR	MSc	**99.7/.999**	35.7/.597								
F	MIR-FT	FossC		**37.3/.611**								
P	MIR	MSc				**99.4/.997**						
P	NIR	AfiLab			*8.2/.284*	*9.3/.304*						
L	MIR-FT	FossC					**98.9/.995**					
L	NIR	AfiLab					*3.8/.194*	*3.3/.181*				
SCC	DC	FossS								**99.1/.995**		
SCC	NIR+Pot	AfiLab							*2.2/-.148*	*2.1/-.143*		
log SCC	DC	FossS										**93.8/.969**
log SCC	NIR+Pot	AfiLab									*9.0/-.3*	*5.4/-.233*

RESULTS AND DISCUSSION

Reference devices were regularly included in the proficiency testing of analytical capability with successful results. The combined expanded uncertainties of measurement results were as follows: ± 2.77 % relative for F (± 0.101 for the original units (%)); ± 2.59 % rel. P (± 0.085 % orig.); ± 2.77 % rel. for L (± 0.115 % orig.); ± 9.3 % at SCC ≤ 900 $10^3.ml^{-1}$. The statistics of reference results (indirect methods) of milk indicators (F, P, L, C, SCC and log SCC) for sets of validation samples (n = 2 for F, P, L, and SCC, for C = 1)) are shown in Tab. I and II. The variation range of the milk indicators shows that the samples were suitable for the validation of the AfiLab measurement abilities (CSN 57 0536). It is clear that variability of reference values needed for adequate validation of the results was achieved (Hanuš *et al.*, 2007) by using of the individual milk samples: – for F from 18.6 to 20.3 %; – for P from 8.4 to 12 %; – for L from 2.9 to 5.4 % (Tab. I and II); – for C 6.9 % (Tab. I); – for SCC from 182 to 204 % (Tab. I and II). In general, there were small differences between reference means (between MSc, LastoSc and FossC) for F, P and L while between reference (MSc, LastoSc and FossC) and AfiLab means the differences were significantly larger (Tab. I and II). This is similar in SCC (Tab. II) where the differences between reference means (between FossS and FossC) were smaller and between reference and AfiLab means large. However, this fact is not essential for evaluation of AfiLab result validation because this shift in values is statistically easy solvable by relevant calibration. Therefore, the mutual relationships between results of analytical methods are more important for such evaluation.

Relations between the reference values (indirect methods, MIR, MIR-FT, DC and DF and direct method Pot) and the values of alternative method (AfiLab) are shown in the Tab. III and IV for observed milk indicators (F, P, L, C, SCC and log SCC). The calculated correlation coefficients (r) were tight and statistically significant ($P \le 0.01$ and ≤ 0.001) mainly between the reference methods each for F, P, L and SCC (Tab. I and II, Fig. 2, 3 and 4). Typical examples of linear regressions are selected in the Fig. 2, 3, 4, 5, 6 and 7. As expected, AfiLab method showed less close relations as compared to reference values. However, these were significant ($P \le 0.01$ and ≤ 0,001) for fat (Tab. III and IV, Fig. 5), r = from 0.597 to 0.738. Similarly, for milk proteins there are less close relations (Tab. III and IV, Fig. 6), r = from 0.284 to 0.787 (P > 0.05 and P≤0.001) when the r value variance was larger than that of fat. There were no significant relationships at lactose (Tab. III and IV) r = from −0.013 to 0.194 ($P > 0.05$). Further, more significantly strong relationship was observed in the electrical conductivity ($P \le 0.001$) as r = 0.773 (Tab. III and IV, Fig. 7). On the other hand the relationship about SCC measuring was not significant ($P>0.05$) as r = from −0.148 to −0.133 (Tab. III and IV) and relevant logarithmic SCC transformation did not improve the nature of this methodical relationship. The variability of reference results explained following proportions of alternative method variability: – for F up to 54.4 % (Tab. III, Fig. 5); – for P to 61.9 % (Tab. III, Fig. 6); – for L only 3.8 % (Tab. IV); – for C up to 59.7 % (Tab. III, Fig 7); – for SCC the worse results could be negatively influenced by the conversion from AfiLab classes. An another reason may be less suitable SCC data distribution within the measured range (Tab. III and IV, Fig. 4).

According to the authors Katz (2007), Katz and Pinsky (2008) and Ishay *et al.* (2011), the AfiLab results may not be as accurate as in the laboratory. This conclusion is confirmed by previous results (Vines *et al.*, 1986; Golc Teger *et al.*, 1996; Golc Teger, 1997; Hanuš *et al.*, 2011 a) regarding the composition of milk. The mentioned fact is accepted particularly in the case of SCC results as evidenced by the results of a series of papers (Vines *et al.*, 1986; Arndt *et al.*, 1991; Heeschen *et al.*, 1994; Hanuš *et al.*, 2011 b; CSN EN ISO 13366−1; CSN EN ISO 13366−2). In contrast to this fact the C results not yet been examined by performing of classical proficiency testing (Grappin, 1993; Leray, 1993, 2007, 2009, Wood, 1994; Fuchs, 2000; Barbano, 2009; Baumgartner, 2009; Castaneda, 2009) although they are frequently used to check the health status of the mammary gland of dairy cows. However, explanatory power of AfiLab results (their reliability) for the animal increases just by the regularity of measurement (real time applications). Karp and Petersson Wolfe (2010) found the determination values of relationship to calibration (indirect) methods for AfiLab 64–76 % for F, 45–52 % for P and 19–52 % for L. It is higher in the case of fat and lactose and lower in case of protein as compared to our results. In general, for demanding conditions of flow milk measurement the values of determination are interesting for practical use.

Said correlation values r (x minus 1.64 × sd for confidence interval (one-sided) at the 95 % probability level; Grappin, 1987) can be used as standards for mentioned alternative analytical method when evaluation of the quality of performed calibrations. If a standard does not specify otherwise these limits should be: – for F 0.564; – for P 0.784; – for C 0.715. The mentioned values of standard deviations of means of individual differences MDsd (x plus 1.64 × sd for confidence interval (one-sided) at the 95 % probability level; Grappin, 1987) can be used as standards for AfiLab method in assessing of the quality of performed calibrations. If a standard does not specify otherwise these limits should be: – for F ± 0.579 %; – for P ± 0.206 %. For instance, at MIR and MIR-FT calibration is the relevant value ± 0.07 % in both cases (CSN 57 0536).

Katz (2007) mentioned two milestones in the automatic data capture type of real time (RT) in rearing of dairy cows and in management of dairy herds respectively. These are electronic flow meter for regular milk yield recording (for breeding purposes and genetic improvement) and activity-meter with electronic identification of dairy cows and their physical activity to ensure reproduction and control of oestrous cycle respectively. These procedures are now almost a classic part of modern milking parlours. Furthermore, the RT AfiLab system then called Katz (2007) as the third milestone in this professional field. In connection with the advent of technology of RT milk analyzes (used in the milking parlour) to dairying for individual milk samples there were also speculations and questions (Rodenburg, 2011) about the possible end of the central milk laboratories in classical milk recording (MR). Those are working 60 years in the mentioned system (MR – 100 years) and use basic dairy analytical reference and indirect methods. This is usually on the system levels (Grappin, 1993; Fig. 1) alpha (as calibration) and beta (predominantly routinely). However, the opposite is true in that regard. Said RT application (AfiLab) strengthens significantly the position of central laboratories in the system of MR as it requires periodic calibrations on level gamma or delta. Katz (2007) and Ishay *et al.* (2011) presented the essence and principles of these periodic calibrations of AfiLab equipment according to the results of monthly MR and also own use of AfiLab procedure in a real environment.

The AfiLab (AfiFarm) offers list of cows suspected from subclinical ketosis disease (according to the dynamics of milk yield and the F/P ratio in early lactation) and dairy cows suspected from subclinical mastitis (according to the dynamics of milk yield, L, C and SCC during lactation) after RT analyses which contributes to the management of dairy herds in terms of promotion of health and reproduction of dairy cows and milk quality (Durkin, 2012). In this context, after 6 years of herd management, Diepersloot (2011) documented and noted progressive radical improvement of dairy herd in the bulk SCC by regular using of AfiLab results from 559 to 167 $10^3.ml^{-1}$. At the same time also average calving interval of cow herd was shortened from 478 to 413 days.

CONCLUSION

As regular transfer of calibrations from indirect methods in laboratories of milk recording on AfiLab is a basic presupposition of system function (Katz, 2007; Ishay *et al.*, 2011), the definition and determination of the previous limit values is important to control the operation of real time analyzes of milk. This is important to know here calculated limits in terms of methodical point of view because of validation procedures and estimations of result uncertainties (CSN EN ISO/IEC 17025). Their importance will increase with further practical implementation of this advanced technology of management of dairy herds.

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