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Organization of Proficiency Testing for Dairy Laboratories in Croatia, Bosnia and Herzegovina and Macedonia in Order to Improve Quality Assurance

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Summary

Participation in proficiency testing is not only an obligation for all analytical laboratories which tend to be credible, but also an opportunity to check how the results agree with the reference or assign value. The Reference Laboratory for Milk and Dairy Products of the Dairy Science Department, Faculty of Agriculture University of Zagreb, is itself incorporated in the proficiency testing organized by dairy laboratories from Germany, Italy, France, Switzerland and Slovenia. The aim is to find out its own accuracy and reliability in particular milk and dairy products analyses. On the basis of seven years experience of participating in proficiency testing, five years ago the Reference Laboratory started organizing its own proficiency testing for dairy laboratories in Croatia, Bosnia and Herzegovina and Macedonia for milk components such as milk fat, protein, lactose and somatic cells count. The results of the analyses have been statistically analyzed and, on the basis of Z-score, the successful measurements have been estimated. The aim of this paper is to demonstrate the organisation and data processing of proficiency testing for milk fat, protein, lactose and somatic cells count in milk for the involved dairy laboratories.

Key words: Proficiency testing, milk, milk fat, protein, lactose, somatic cell count

Introduction

Food quality control, including the control of milk and dairy products as well, is essential for health benefits in every country and it is well described in legislation. The legislation includes not only a catalogue of components and their quantities allowed (or forbidden) in certain foodstuff, but also a register of the authorized institutions for their analyses.

According to the Croatian National Milk Control Regulation (Croatian National Milk Quality Regulation, 2000) Croatia, like other countries, also has a list of demands on milk quality and established criteria for the Reference Laboratory and the National Central Laboratory for Milk. The latter, in accordance with the European regulations, controls (milk) samples of every milk producer in Croatia, whereas the Reference Laboratory for milk and dairy products prepares secondary reference material for cow, sheep and goat milk for analytical instruments calibration in local dairy industry and for the National Central Laboratory's routine work control. In

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that way, by establishing an independent milk quality control system, Croatia is on an equal footing with the other European countries.

The Reference Laboratory itself is incorporated in the proficiency testing (PT) organized by accredited (ISO/IEC 17025:2005) laboratories in Germany, Italy, France, Switzerland and Slovenia. The aim is to find out its own accuracy and reliability in a particular milk and dairy products analysis.

On the basis of seven years experience of participating in proficiency testing, the Reference Laboratory started with the organization of PT for milk fat, protein, lactose and somatic cells count in Croatia and some other countries in the region, so, in that way extended its control from some local and the National Central Laboratory to further 14 laboratories. Management (statistical analysis) of the proficiency testing is based on the *"International Harmonized Protocol for Proficiency Testing of Chemical Analytical Laboratories"* (Thompson et al., 2006). The results of the analyses are statistically analyzed and, on the basis of Z-score, the successful measurements are estimated.

Milk quality depends on numerous variable parameters which have to be determined. Frequently, there are several methods for each parameter analysis and, in this way, minor distinctions are usually found in the results. For example, milk fat can be determined by gravimetric reference method (Röse-Gottlieb), butyrometric method (Gerber) and by MID infrared instruments. Standard methods for determination of chemical components and hygienic quality (somatic cell count and overall bacteria count in milk) are crucial for estimating the milk price. Thus, quality assurance becomes necessary to ensure that the analytical results are accurate. One of the crucial elements of every quality assurance manual is the participation in proficiency testing (ISO/IEC 17025).

The aim of this paper is to demonstrate the organization of PT for milk fat, protein and lactose analysis, as well as somatic cells count in milk for different dairy laboratories using MID infrared instruments and fluoro-optoelectronic counters in Croatia, Bosnia and Herzegovina and Macedonia.

Materials and methods

Individual cow milk samples for the PT have to be representative for all chemical components, ranging from minimum to the maximum values. The number of somatic cells and overall bacteria count in milk samples should comply with the Croatian National Milk Quality Regulation (2000).

Since 2005, the Reference Laboratory has been accredited according to ISO/IEC 17025:2005. Secondary reference material for the PT is prepared once a month which requires 20 to 25 high quality cow milk samples in Reference laboratory. The quantity of samples depends on the number of registered participants in PT. An average number of participants is 20, and therefore about three liters of milk per sample is needed.

Since milk fat, protein and lactose content as well as somatic cell count determination are of interest, firstly the samples are analyzed by the routine methods to get faster insight in milk quality and content, because samples must comprise a specific content range. Milk samples are analyzed by the infrared spectroscopy (IR) method (using the instrument Milkoscan FT 120, according to ISO 9622 (1999) to determine milk fat, protein and lactose content, the fluoro-opto-electronic method is used to determine somatic cell count (using the instrument Fossomatic Minor, according to ISO 13366-2 (2006), whereas the flow citometry method is used to determine an overall bacteria count (using the instrument Bactoscan FC, according to ISO 21187 (2004). Based on obtained results, 7 samples are chosen for PT for milk fat, protein and lactose using IR instruments as well as 7 samples for somatic cell count instruments using the fluoro-opto-electronic method. After that, chosen samples are preserved with bronopol (except samples for somatic cell count which are preserved with azidiol), and as soon as the preservative is dissolved, samples must be uniformed (homogenized). For homogenization of milk samples agitator (Reax 20, Heidolph, Germany) is used. Successfulness of homogenization is checked by 5 repeated measurements of every sample on the IR instrument. If standard deviation of repeatability (S_r) is higher then 0.03 g/100 g for milk fat, protein and lactose (determined by validation of the IR method) and for somatic cell count coefficient of variation (CV) is higher then 6 % (determined by validation of the method) sample will not be used for PT. Then the samples are portioned into bottles of approximately 100 millilitres. Each participant of PT gets one set

which consists of 7 samples, whereas the Reference Laboratory keeps three equal sets. These sets are used for reference values for milk fat, protein and lactose content determination as well as somatic cell count. Reference values (X) are determined by the reference methods (ISO 1211:1999, ISO 8968-2:2001, ISO 5765-1:2002, ISO 4833:2003, ISO 21187:2004).

The validity of preserved milk samples is 5 days since preparation (determined by validation of method). In the next three days all three sets are analyzed (new set each day) in 5 parallel determinations. Reference values are determined as average of 15 measurements of each component. S_r must be within values determined by validation. S_r for milk fat is ±0.007 g 100 g^{-1}, protein ±0.004 g 100 g^{-1}, lactose ±0.011 g 100 g^{-1} and for somatic cell count maximum CV must be less than 10 %. S_r of reference methods are higher then those of IR routine method (homogenization) because of their higher sensitivity and accuracy. If results are not complied with values mentioned above, the sample is discarded from the set.

The prepared milk samples are kept in the refrigerator (5±3 °C) until they are sent over to PT participants (within two days). In transportation of the samples, a portable refrigerator (5±3 °C) is used and participants must keep the samples under the same conditions until further analyses. The analysis procedure is as follows: first all samples must be left until they gradually reach the room temperature, then they have to be heated in a water bath until they reach 38-42 °C, according to the instructions of the manufacturer for MID infrared instruments (ISO 9622:1999). On that temperature range, the sample is completely homogeneous. Before analysis, the samples have to be gently shaken. The Validation of methods has shown that the mentioned temperature range has no significant influence on the results. When PT participants' results are received, statistical analysis is made to compare these results with reference values obtained in the Reference Laboratory. The criterion for the choice of laboratory has been made by most frequent participation in PT.

Statistical analysis

The statistical analysis was performed according to the AOAC Protocol (Thompson et al., 2006). The first stage is calculating bias, i.e. the difference between laboratory results and reference value. Hence:

$$\text{bias} = a - X$$

where: X - reference value or, in practice, the best estimate of X. This value is determined by reference method; a - measured value obtained by laboratory (participant of PT).

Next step is comparison of the bias estimation with a target value for standard deviation that forms the criterion of performance.

Z-score is formed as follows:

$$Z = (a-X)/\sigma$$

where: σ - standard deviation of PT assessment.

As Z is standardized, it is comparable for all components and methods. Z scores are combined to give a composite score for a laboratory in single cycle of PT (Figure 2-5).

The Z-scores can therefore be interpreted as follows:

$|Z| \leq 2$ result is satisfactory

$2 < |Z| < 3$ result is questionable

$|Z| \geq 3$ result is unsatisfactory

Unfortunately, since all our PT participants do not express measurement uncertainty of their results, we can not establish zeta score (**ξ**) and, therefore, give better estimation of results. The zeta score provides an indication of whether the participants' estimation of uncertainty is consistent with the observed deviation from the assigned value (Thompson et al., 2006).

Results

Out of 17 laboratories which participated in PT, five of them have been chosen for the demonstration of chemical components and three for the somatic cell count. For each laboratory involved in a PT, an annual report was prepared. According to the annual results of participation in PT, participants can conclude about quality of their own performance and determine possible presence of systematic error. Figure 1 shows the laboratory that, during the defined period of time, had a slight oscillation of Z-score, which can be described as random error.

Figure 1: Z-score averages for milk fat during 2003 to 2007
Grafikon 1: Srednje Z-vrijednosti za mliječnu mast od 2003. do 2007. godine

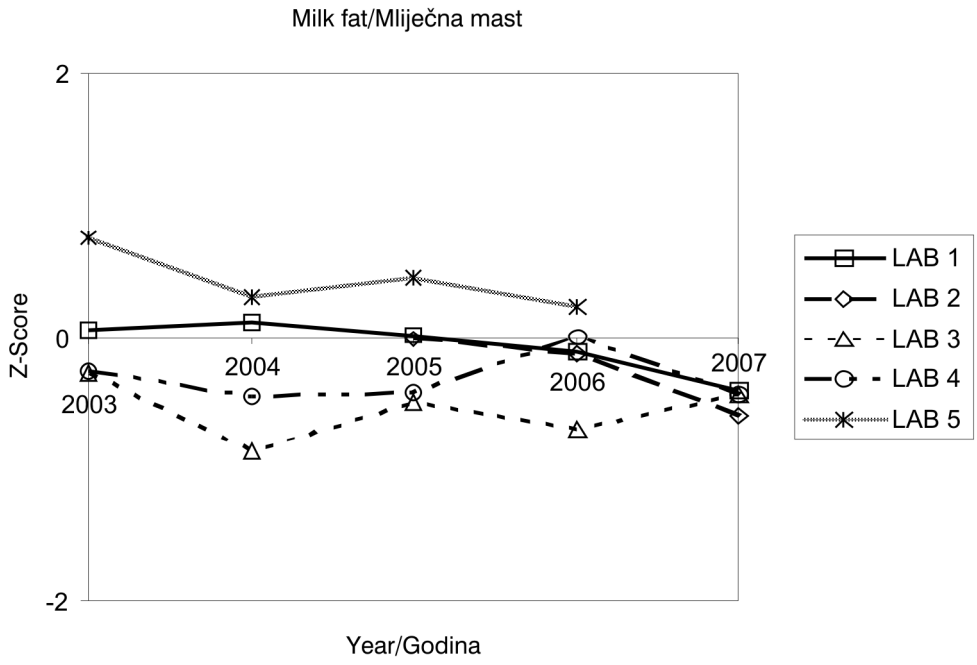


Figure 2: Z-score averages for milk protein during 2003 to 2007
Grafikon 2: Srednje Z vrijednosti za proteine od 2003. do 2007. godine

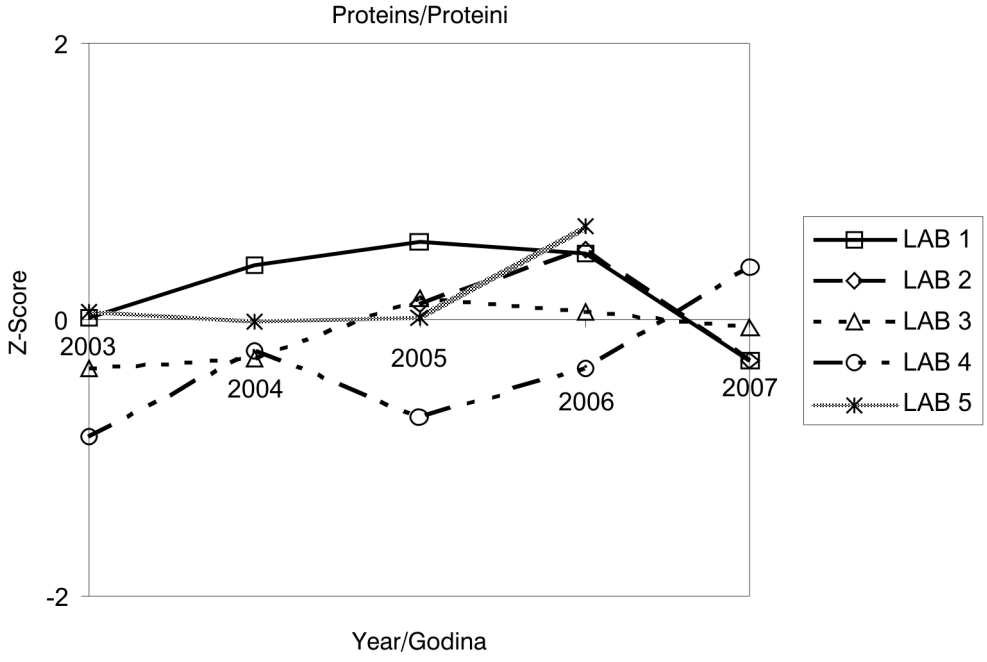


Figure 3: Z-score averages for lactose from 2003 to 2007
Grafikon 3: Srednje Z-vrijednosti za laktozu od 2003. do 2007. godine

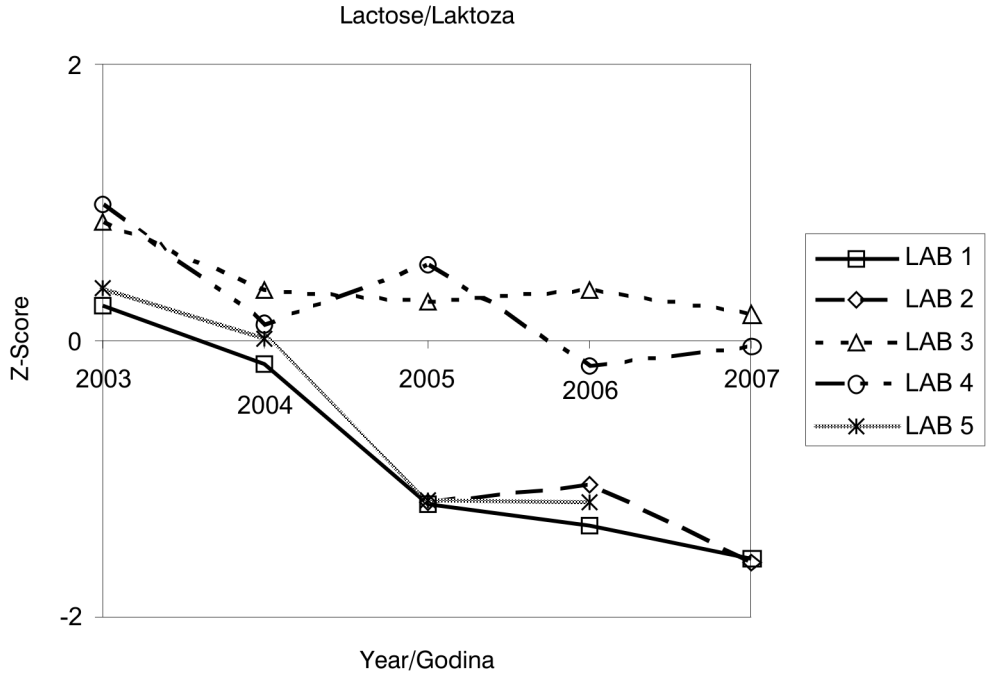
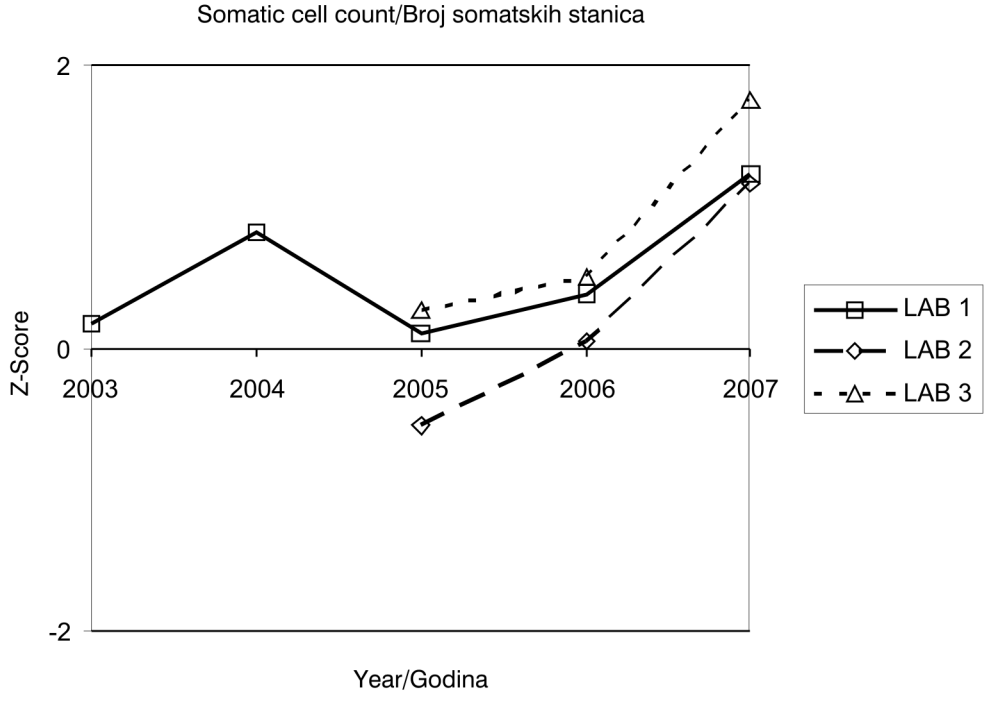


Figure 4: Z-score averages for somatic cell count during 2003 to 2007
Grafikon 4: Srednje Z-vrijednosti za broj somatskih stanica od 2003. do 2007. godine



Figures 1-4 show the trend of Z-score towards different milk components. It refers to how successful all participants have been in analyzing milk fat, protein and lactose and somatic cell count during the years of participation. The Z-score for every referring year is an average of twelve annual PTs. Thus, all laboratories whose results are presented in Figures 2-5 have maintained their results within the satisfactory range but their trends can be followed. That type of report was not sent to participants but it was used for our own control.

Figures 1 and 2 indicate that all chosen laboratories have maintained their Z-score within the range of -1 and +1 which is a very good performance.

Figure 3 shows that laboratories 3 and 4 had stable performance and, in time, they have approached the reference value (zero on the graph). At the same time, laboratories 1, 2 and 5 have indicated the decrease of performance quality (negative trend). That is probably because of some sort of systematic error caused by poor calibration, poor homogenization of the samples, new and inexperienced staff. We can assume that the laboratories 1, 2 and 5 will improve their performance when/if they reach Z value under -2.

PT for somatic cell count started in 2003 with one laboratory and, in time, the number of participants has increased. Figure 4 shows a similar trend of chosen laboratories since 2005. As opposed to lactose (Figure 4), in time, laboratory performances have increased the distance from reference value (positive trend). The most probable cause is wrong preparation of dye solution.

Conclusion

One of the most important ways for quality control of laboratory performance is the participation in proficiency tests. In that way, the accuracy of measurement can be checked. All presented laboratories show results within $-2 \leq Z \leq 2$ but some of them have negative or positive trend towards limit values of Z. Although, all participants are within the range, the trend of particular laboratories shows their systematic error. This trend can lead to questionable results in the future. A long-term goal of the PT is the establishment of the competence of the tested laboratories. Since we have already asked PT participants to send us their measurement uncertainties, statistical analyses will be done by ξ-score in the near future.

Organizacija ispitivanja osposobljenosti za mljekarske laboratorije u Hrvatskoj, Bosni i Hercegovini i Makedoniji u cilju boljeg osiguranja kvalitete

Sažetak

Sudjelovanje u ispitivanju osposobljenosti (ring test) nije samo obveza za sve analitičke laboratorije koji nastoje biti vjerodostojni, nego predstavlja i mogućnost provjere podudarnosti rezultata sa referentnom vrijednošću. Referentni laboratorij za mlijeko i mliječne proizvode Zavoda za mljekarstvo Agronomskog fakulteta Sveučilišta u Zagrebu uključen je u ispitivanje osposobljenosti koje organiziraju mljekarski laboratoriji u Njemačkoj, Italiji, Francuskoj, Švicarskoj i Sloveniji. Namjera ispitivanja je provjera vlastite točnosti i pouzdanosti izvođenja analiza mlijeka i mliječnih proizvoda. Na osnovi sedmogodišnje uključenosti u ispitivanje osposobljenosti, prije pet godina Referentni laboratorij započeo je s organiziranjem ispitivanja osposobljenosti za mljekarske laboratorije u Hrvatskoj, Bosni i Hercegovini i Makedoniji, za pojedine sastojke mlijeka kao što su mliječna mast, proteini, laktoza te broj somatskih stanica. Rezultati analiza statistički se obrađuju i na osnovi Z-vrijednosti procjenjuje se uspješnost mjerenja (izvođenja analize). Cilj rada je prikazati organizaciju i obradu rezultata ispitivanja osposobljenosti za mliječnu mast, proteine, laktozu i broj somatskih stanica za mljekarske laboratorije uključene u ispitivanje.

Ključne riječi: ispitivanje osposobljenosti, mlijeko, mliječna mast, proteini, laktoza, broj somatskih stanica

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