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Maisons-Alfort laboratory for food safety

EURL MMP
European Union Reference Laboratory for Milk and Milk Products

Report of the 15th workshop of the NRLs for Milk and Milk Products

3-5 October 2012, Maisons-Alfort

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1 OPENING :

Laurent LALOUX, Director of the European Union Reference Laboratory for Milk and Milk Products (EURL MMP) at the ANSES Maisons-Alfort Laboratory for Food Safety, opened the meeting on Wednesday at 2.30 pm and welcomed the participants.

Bertrand LOMBARD, EURL MMP Manager, introduced the meeting. He was glad that at least one NRL representative from all EU Member States (MSs) took part to the workshop.

Roll-call of delegates

Each delegate introduced itself (see the list of attendance, appended). 28 NRLs from 25 EU Members States (MSs) and from Norway and Switzerland were represented. Each delegate introduced itself (see the list of attendance, in annex). Apologies from Christer WIBERG (SLV, SE), Klytaimnistra VELETA (GR), Laust ØSTERGAARD (DK) were received.

Klaus KOSTENZER, in charge of milk hygiene and follow-up of the EURL MMP at EC/ DG SANCO Health & Consumers, attended the meeting on Thursday.

All additional documents (i.e. agenda and presentations) have been provided to the participants on USB sticks and are available online upon request.

2 GENERAL TOPIC

2.1 ACCREDITATION OF THE NRLS

Adrien ASSERE presented an overview of the accreditation status of the NRLs, based on the outcome of an enquiry launched on 30/08/2012.

He pointed out that some efforts were still needed for NRLs to finalise their accreditation process on the reference methods prescribed by the EC Regulation EC 2074/2005 modified. Indeed only 12/32 and 14/32 were accredited respectively on the Standards EN ISO 11816-1 (alkaline phosphatase activity in milk) and EN ISO 13366-1 (somatic cell counting in milk).

Bertrand LOMBARD noticed that accreditation is a resource-consuming process, it is not easy to maintain for methods rarely used in routine and, for reference labs, may be more suited for validation process (flexible scope).

Klaus KOSTENZER noted the tricky situation that about half labs were not accredited for 2 analyses of the EURL/NRL scope with reference methods, and clarified that it was the role of Food and Veterinary Office (EC inspection organization) to follow this aspect in each Member State (MS). He also agreed that subcontracting of certain analyses by an NRL to another lab was possible, the NRL remaining the contact point for the EURL.

2.2 NATIONAL SITUATION OF MILK PRODUCTION

Koen de REU (ILVO, BE-NRL) presented the Belgian situation of milk production, control and hygiene. See his slides.

Several questions were focused on the way to handle non-conformities to limits (penalties)

➔ The EURL would launch an enquiry to NRLs on situation of milk production and control at national level.

2.3 PROFICIENCY TESTING TRIAL ORGANISATION

Bertrand LOMBARD introduced this topic, being one of the main duties of NRLs at national level.

2.3.1 IN SLOVENIA

Lena HODOSCEK (NVI, SI-NRL) presented the activity undertaken by another institute (University of Ljubjana, Institute of Dairy Science and Probiotics) that organises PT trials on Total Flora (TF) and Somatic Cell Counts (SCC) for 8 Slovenian official laboratories.

Bertrand LOMBARD reminded that subcontracting the NRL PT trials was possible; at the condition that the NRL be informed of individual results. The follow-up of labs having obtained deviating results still remains a task of the NRL.

Some NRLs follow the homogeneity/stability of the samples for TF or SCC with analyses of fat content. This approach was not recommended by the EURL, because, in case of satisfactory homogeneity, there would be no evidence that the analyte (TF or SCC) would behave in the same way.

2.3.2 IN BELGIUM (ILVO)

Koen de REU presented the PT trials organised by ILVO for milk payment laboratories and for milk industry laboratories. ILVO is accredited for PT organisation.

➔ In conclusion of this session:

1/ the EURL would launch an enquiry on practices of NRLs which organize PT trials (in particular sample preparation, homogeneity & stability testing).

2/ B. Lombard would present at the next workshop CEN ISO/TS 22117, containing specific guidance for PT trials in food microbiology, applicable to TF in raw milk.

3/ the EURL would later draft a guide for the NRLs on PT organization on TF & SCC in raw milk (especially on sample preparation, homogeneity & stability testing).

3 HYGIENE OF RAW MILK

3.1 SAMPLE STUDIES FOR PTS

3.1.1 STUDIES OF COW'S MILK USED FOR PT ON SOMATIC CELL COUNTS (2012)

Alexandra CAUQUIL (EURL MMP, Unit EDB) presented the outcome of this study.

The PT trial for the NRL would be organised in October 2012 (see 3.2.2).

3.1.2 STUDIES OF RAW GOAT'S MILK SAMPLES USED FOR PT TRIAL ON TOTAL FLORA ENUMERATION

Rabeb MILED (EURL MMP, Unit EDB) presented this study conducted for the forecasted 2013 PT trial.

Some NRLs suggested to pay attention to high SCC levels in goat's milk which may affect the robustness of the TF method.

3.2 PT TRIALS

3.2.1 2011 – TF IN GOAT'S MILK

Rabeb MILED presented the outcome of the PT trial organised in 2011 dedicated to TF in goat's milk. The global performance of the network of NRLs, both in terms of repeatability and reproducibility, was good. Moreover, most of the participants (81 %) showed a satisfactory individual performance, in terms of precision (k-ratios) and trueness (z-scores).

3.2.2 2012 – SCC IN COW'S MILK

Alexandra CAUQUIL briefly presented the design of the forthcoming (October 2012) inter-laboratory PT trial dedicated to SCC in raw cow's milk by the reference method, the Standard EN ISO 13366-1.

3.3 METHODS

3.3.1 COMPARISON BETWEEN PCA AND PCA +MILK FOR THE ENUMERATION OF TOTAL FLORA IN MILK

Rabeb MILED presented the outcome of available studies on this topic and an additional study performed at the EURL on goat's milk. The majority of results of studies comparing PCA & PCA+milk show concordance between these 2 media for TF enumeration in goat's and cow's milk. The EURL comparison study on goat's milk should be further conducted with more samples for statistical analyses.

Hans-Georg WALTE (D-NRL) also presented a study comparing the PCA+milk media from 5 different manufacturers showing significant differences between the different manufacturers. The manufacturer of the media may impact the PCA or PCA+milk performance, which reflects a more general situation in microbiology.

➔ It was agreed that the EURL would associate volunteering NRLs to this comparative study. This study would focus on liquid milk. Upon recommendation of L. HODOSCEK, the participants may enumerate the plates after 48h & 72 h incubation (to compare the 2 incubation times and to investigate whether 48 h may be sufficient). Results would be collected 1 month before the next workshop, to present a synthesis of this study at the next workshop.

3.3.2 THE CONVERSION RELATIONSHIP IN RAW GOAT'S MILK FOR THE ENUMERATION OF TOTAL FLORA AT 30°C

Rabeb MILED presented the progress of this study. The Bactocount settings still needed to be optimised for TF enumeration in raw goat's milk.

Upon suggestion of Martin ALEWIJN (RIKILT, NL-NRL), the whole Bactocount spectrum would be investigated in collaboration with the manufacturer, Bentley.

3.3.3 STUDY OF IMPACT OF FACTORS INFLUENCING THE CONVERSION RELATIONSHIP IN COW'S MILK

Alexandra CAUQUIL presented shortly the conclusion of the 1st phase of the study led on that topic (technical report dispatched by circular letter of 16/01/2012, paper submitted) and explained the experimental design of the 2nd phase of the study where different important factors were selected.

To reply to Bianca PONGRATZ and Birgit ROSSMANN (AGES, AT-NRL), Véronique DEPERROIS (EURL MMP, Head of EDB Unit) explained that feed was not selected as a studied factor, because it is included in the factor "season" (winter-summer, having a direct impact of the nature of feeding). Giuseppe BOLZONI specified that the feed is an indirect factor of the faeces contamination that could contaminate the udder.

3.3.4 IDENTIFICATION OF TOTAL FLORA IN RAW GOAT'S MILK BY MOLECULAR TOOLS

Rabeb MILED presented the progress of study undertaken at the EURL on the identification of flora by PCR-DDGE / TTGE.

To reply to a question of Hans-Georg WALTE, Rabeb MILED explained that these methods are able to provide also a partial idea of the quantity of the different flora present.

3.3.5 COORDINATION - HARMONISATION

Conversion relationship (CR) between instrumental and reference method for TF in raw milk

Bertrand LOMBARD presented the outcome of the meeting on 3rd October morning of the working group (WG) on harmonization of CR between instrumental and reference method for TF in raw milk. The WG, convened by Veronique DEPERROIS, agreed to conduct a feasibility study to harmonize CR at European level. Next WG meeting in would take place in 2013 when data would be collected and statistical analysis done.

Implementation of EN ISO 21187

➔ Instead of drafting a document on practical guidance to implement ISO 21187, as envisaged in the past, it was agreed that the EURL would collect from NRLs proposals to revise this standard, whose revision would be soon launched by ISO/IDF.

Revision of check-list for lab visit on establishment of CR

➔Jolanta ROLA (PL-NRL) would send to EURL a proposal.

3.3.6 VALIDATION CRITERIA OF INSTRUMENTAL METHODS FOR TF AND SCC

Véronique DEPERROIS presented a first proposal of criteria for the validation of instrumental (epifluorescent) methods for the enumeration of somatic cells in raw cow's milk, sent by circular letter on 24/09/2012.

It was clarified, that since EC Regulation 2074/2005 modified refers to ISO 8196 and ISO 13366-2 which do not require a validation of alternative method by inter-lab study, the latter could not be required in the EURL document, but could be recommended.

➔ The EURL would modify the draft according to the meeting, send it to IDF/ISO for comments, in order to finalize it in early 2013.

3.3.7 CERTIFICATION OF INSTRUMENTAL METHODS FOR TF AND SCC

Bertrand LOMBARD gave an overview of the on-going process undertaken by certification bodies of instrumental methods (Bactocount, Bactoscan) for TF in raw milk.

SCC would be the next step. The EURL would send the finalized EURL document on validation criteria (3.3.3.3) to certification bodies (AFNOR Certification, MicroVal) in view of certification of instrumental methods (at first Foss & Bentley's instruments) by end of 2014.

3.3.8 UPDATE ON THE REVISION OF IDF161A

Véronique DEPERROIS provided an update on the revision of IDF 161 A (ISO/DIS 16297) Standard on the protocol for the evaluation of alternative methods for TF in raw milk: the draft for final vote (FDIS) was being prepared by IDF/ISO group S07.

3.4 CRITERIA FOR COLOSTRUM: SECOND ENQUIRY

Rabeb MILED presented briefly the new enquiry on colostrum that would be sent soon to NRLs. She would also undertake a new review of literature.

Klaus KOSTENZER noted that a model for certificate to import in EU colostrum and colostrum-based products from third countries: he would need EURL opinion on what criteria to apply.

3.5 REFERENCE MATERIALS

3.5.1 DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS (CRM) FOR SCC

Véronique DEPERROIS provided the update on the topic. This year the EURL had 2 meetings with EC/IRMM-JRC Geel, IDF/ICAR and DG SANCO. To take the decision to develop CRM for SCC, JRC needed a feasibility study to prepare reference materials with lyophilized milk, to be conducted by the EURL, and also an estimate of the market needs, to be conducted by IDF/ISO and EURL, through an enquiry to NRLs.

Thomas BERGER (ALP, CH-NRL) confirmed the need of CRMs in this field.

To answer a question of Martin ALEWIJN, Bertrand LOMBARD specified that the timeframe could be 3 to 4 years in case every step is going positively.

Belgian and Dutch NRLs proposed that PT trials organized by EURL on SCC be more frequent than on TF because of the current lack of CRMs and the lack of commercial PT schemes on SCC using the reference method.

3.5.2 REFERENCE SYSTEM FOR SCC

Thomas BERGER (ALP, CH-NRL) gave an update of the progress of work within the IDF/ICAR WG.

3.6 NRLS TRAINING

Alexandra CAUQUIL and Rabeb MILED presented briefly the 1st training session organised earlier in 2012 on SCC. A 2nd session would be organized in 2013.

3.7 PROPOSALS FOR 2013 WORK PROGRAMME

The EURL had to send to DG SANCO a proposal for 2013 work programme prior to the workshop (by end of August), consisting of the following actions (also extending beyond 2013 for some of them):

- Inter-laboratory PT trials:
  - Development of samples for PT (TF and SCC in cow's milk and TF in sheep's milk)
  - PT trial on SCC (and not TF as initially proposed to DG SANCO) in raw cow's milk
- Methods:
  - Comparison of PCA+milk/PCA agars: continuation
  - TF with instrumental method (Bactocount) in cow's & goat's milk: continuation
  - Harmonization of conversion relationship between instrumental and reference methods for TF in raw milk: continuation of WG
- Reference Material:
  - Development of CRM for SCC: collaboration with JRC, feasibility study and enquiry to NRLs
  - Somatic cells' slides and pictures to identify and count somatic cells prepared for NRLs
- Training:
  - SCC

4 PASTEURISATION TRACERS

4.1 INTRODUCTION

Marina NICOLAS (EURL MMP, Team CAT-AP) introduced the work area on pasteurisation tracers, presenting the topics of the workshop. She explained in particular the reasons for the delay in the work scheduled linked to human resources problems.

Sharon CASSIDY (UK-NRL) raised the need to test alkaline phosphatase (AP) in butter and the possible setting up of a legal limit for butter. Ciara O'DOWD (IE-NRL) raised also a question on the possible need of separate limits for AP in cream and whey, when they are pasteurized separately. Klaus KOSTENZER indicated that this was not in the EURL work programme, at least for 2013 but it may be envisaged later.

4.2 FIXATION OF AP LEGAL LIMITS IN PASTEURIZED GOAT MILK

Bulgarian NRL

Borislav KASHAMOV (NVMS, BG-NRL) presented his study on pasteurised goat milk. Data showed that the values for AP activity in Bulgarian pasteurized goat milk comply with the proposed limit of 350 mU/l.

Outcome of European study

The EURL would draft a report based on results obtained by NRLs.

4.3 MILK FROM OTHER SPECIES

Activity of indigenous enzymes and contents of soluble whey protein of raw and heat-treated camel milk

Peter Chr. LORENZEN (DE-NRL) presented the study conducted in collaboration with the Dubai CVRL laboratory. Several heat-treatment markers were investigated, in alternative to AP which revealed to be not satisfactory for camel milk. According to Peter LORENZEN, lactoperoxidase appeared to be a promising marker, but the method used was a qualitative one.

4.4 STATE OF THE ART AT THE INTERNATIONAL LEVEL

- Revision of EN ISO 11816-1 (AP in milk): under DIS vote
  Hanène GHEZZAL (EURL MMP, Team CAT-AP) underlined the importance of the collaborative trials organized in 2008 & 2010 by the EURL with the participation of NRLs on cow and goat milk, which had provided precision data for the Standard method. She presented the new approach recommended by the IDF/ISO statistical group to express precision data of AP activity.
- Revision of EN ISO 11816-2 (AP in cheese): DIS vote to be launched soon.
- Collection of AP data in cheeses from pasteurized milk: the project is on-going to explore the possibility of a limit at international level. Presently, input is mainly done from EU countries.
- Other heat-treatment tracers:
  At the ISO-IDF level, a new Work Item has been adopted within the Work Program of the relevant Working Group for the development of an analytical method to quantify the Gamma-glutamyl transpeptidase (GGT), an alternative thermal enzyme marker for dairy products where AP has not shown to be a pertinent indicator(ex: camel milk).
- Reactivated & microbial AP: need for reference material.

4.5 PT TRIAL ON ALKALINE PHOSPHATASE IN COW MILK

Hanène GHEZZAL presented the PT trial scheduled in November 2012. The PT would be dedicated to AP activity in cow milk: 3 types (whole, semi-skimmed and skimmed) and 3 target levels of AP per type of milk (a total of eighteen samples).

4.6 PROSPECT ON THE DEVELOPMENT OF AN AP "CERTIFIED" MATERIAL

Martin ALEWIJN presented preliminary work conducted by the Dutch NRL in view of the production of a Reference Material.

Stability was still an issue to tackle.

Dutch NRL and EURL will work together on the organization of the experiments and relevant statistical aspects before the NRL starts a new series of experiments.

4.7 EQUIVALENCE (OR NOT) OF THE CHEMILUMINESCENT METHOD VERSUS THE OFFICIAL FLUORIMETRIC METHOD

Marina NICOLAS presented work done by EURL as regards the validation of an alternative method, the chemiluminescent method, to determine AP activity. The EURL had implemented the approach of accuracy profile (acceptability limits) to assess the equivalence of the chemiluminescent method *versus* the official reference fluorimetric method.

Comparison dealt with cow's whole milk.

The statistical evaluation of the results showed that the 2 methods under study were not equivalent and that results obtained with each method were not correlated by a linear regression.

➔Consequently, the chemiluminescent method cannot be declared as an alternative to the fluorimetric method, in the frame of Regulation 2074/2005 modified. In addition, it cannot even be used as a screening method, since the Regulation mentioned has not defined screening controls for AP.

4.8 FIXATION OF AP LEGAL LIMITS FOR CHEESES MADE FROM PASTEURIZED COW MILK

4.8.1 PROSPECTS FOR THE ESTABLISHMENT OF AN EU REGULATORY THRESHOLD FOR COW MILK PASTEURIZED CHEESE

Luisa PELLEGRINO (expert from University of Milan, IT) and Marina NICOLAS presented to the group a joint work on determination of AP activity as a tool to characterize cheeses made from pasteurized cow milk (presented at the 2011 IDF Summit).

Main points dealt with choice and optimization of the analytical method, a feasibility study on cheese production under known conditions, results on commercially available samples and identification of some problematic cases (blue and mozzarella cheese).

The collection of AP data needs to be enriched by contributions of more EU countries and to be broadened to the international level.

4.8.2 INPUT FROM NRLS

Sharon CASSIDY (UK-NRL), Lena HODOSCEK (SLO-NRL), Martin ALEWIJN (NL-NRL), Giuseppe BOLZONI (IZSLER, on behalf of IT-NRL), Tiina RITVANEN (FI-NRL) and Ida BELICZAY (NEBIH, HU-NRL) presented studies conducted at the national level to determine AP content in cow pasteurized cheese so as to evaluate compliance with the tentative limit proposed by EURL (10mU/g).Almost all of the results presented confirmed this tentative limit (higher levels observed in some cases by the Finnish and Italian NRLs seemed abnormal and need to be further investigated).

4.9 PROPOSALS FOR 2012 AND 2013 WORK PROGRAMME

The EURL had to send to DG SANCO the proposal for the 2013 work programme prior to the workshop (by end of August), consisting of the following actions (also including some actions to be conducted by the end of 2012 or extending beyond 2013):

- PT trial on AP in cow milk, in the range 125-325 mU/L (end 2012)
- Determination of AP in cheese by the fluorimetric method:
  - Preliminary study on 2-3 samples, to evaluate the impact of sample preparation, in collaboration with NRLs or expert laboratories recognized for their proficiency in this method
  - Validation study & PT trial/
- AP in cheeses made from pasteurized cow milk: coordination of national investigations undertaken by (by end 2013);
- ISO/IDF standardization work on heat treatment tracers:
  - revision of Standard EN ISO 11816/IDF 155 parts 1 and 2;
  - project on collection of AP data in pasteurized cheese at the international level (project leader);
  - other tracers of heat-treatment: development of an analytical method to quantify the Gamma-glutamyl transpeptidase (GGT)
- Follow up of work conducted at the international level
- AP inactivation study in ewe's milk
- NRL training: upon request.

5 CLOSURE

B. LOMBARD closed the meeting on Friday at 1:00 pm, hoping that it met the NRL expectations. He thanked all the attendees for their participation and active contributions to the workshop.