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Maisons-Alfort laboratory for food safety

EURL MMP
European Union Reference Laboratory for Milk and Milk Products

Report of the 14th workshop of the NRLs for Milk and Milk Products

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1 OPENING : MONDAY 2ND MAY, 9.15 AM

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 and welcomed the participants.

L. LALOUX gave some news (see his slides) about the changes that took place for our Laboratory: the new agency ANSES (**French Agency for Food, Environmental and Occupational Health Safety**) resulting of the merging of Afssa with Afsset, another change in the EURL MMP organisation is the creation of a new unit Ecophysiology and bacterial detection (EDB) resulting from the merging of the former unit Hygiene and Microbiology of Food Products (HMPA) and a team of the former unit Microbiological Safety in Food Catering & Industrial Processes (SRPI).

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. He welcomed Klaus KOSTENZER, the new representative of EC/ DG SANCO Health & Consumer Protection replacing Paolo CARICATO for the follow-up of the EURL MMP activities.

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). Excuses from Christer WIBERG (SLV, SE), Carmen BLANCO VIDAL (CNA, ES), Oto HANUS (VUCHS, CZ) and Heli REINET (VFL, EE) were received.

All additional documents (i.e. agenda and presentations) are available on the EURL website:

http://crl.lerqap.free.fr/espace/?key=6bb2989cb0962db4e23f93935d0ed918

2 GENERAL TOPICS

2.1 DG SANCO UPDATE

Klaus KOSTENZER presented this topic (see his slides).

2.1.1 EURL EVALUATION

Klaus KOSTENZER presented the process of evaluation of the EURLs by Civic Consulting and the outcome for the EURL MMP which was satisfactory (general mark B+), with some recommendations for improvement (web-site, web-forum). The evaluation report would be soon published.

Klaus KOSTENZER and Bertrand LOMBARD thanked the NRL network for the quality of the cooperation and for their support.

Answering to Bernadette HICKEY, Klaus KOSTENZER explained that the question of the possible extension of the EURL/NRLs MMP mandate had not been yet discussed at DG SANCO level.

2.1.2 AMENDMENT TO EC REGULATION 2073/2005 FOR ENTEOBACTERIACEAE

Further to a request from the EURL and NRL network in the past for improving practicability of analyses in routine controls, the EC Regulation 2073/2005 on microbiological criteria had been amended last year (EC Regulation 365/2010) to allow for the use of the colony count technique for enumeration of *Enterobacteriaceae* (Standard ISO 21528-2) in pasteurised milk and other pasteurised liquid dairy products (criterion 2.2.1).

2.2 PROFICIENCY TESTING ORGANISATION

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

2.2.1 EN ISO 17043 / EN ISO 13528

Soraya AMAROUCHE, LNE, Paris (France) had been invited to present the new Standard EN ISO 17043 (replacing the ISO Guide 43). This standard gives the general requirements for the organization of proficiency testing (PT) trials. This Standard is to be used with the Standard ISO 13528 providing the statistical methods (only for quantitative determinations), also in combination with CEN ISO/TS 22117 specific to food microbiology.

Marina NICOLAS, CAT Unit, EURL MMP, mentioned that it is difficult to comply with the 0,3 factor (corresponding to a variability around 10%) in the homogeneity and stability studies, when applying ISO 13528. Soraya AMAROUCHE replied that taking a variability of 20% could be acceptable.

To answer a question of Koen de REU on the possible requirement of accreditation for PT organization for EURLs/NRLs, it was pointed that it is not in the Regulation 882/2004 on official controls, including duties and requirements for EURLs/NRLs. This may be envisaged for EURLs but it would be clearly too heavy for NRLs.

2.2.2 NRL ACTIVITIES ON PT TRIALS

2.2.2.1 GERMAN NRL

Karin KNAPPSTEIN presented the PTs organised at the Max Rübner-Institut (DE-NRL): 1 PT dedicated to TF enumeration by Bactoscan FC for milk payment/food hygiene laboratories is organised each year and 1 PT dedicated to SCC in raw milk by routine methods is organized each year.

2.2.2.2 BELGIAN NRL

Koen DE REU presented the PTs organised by his laboratory, ILVO-T&V (BE-NRL). These PTs are organised for milk payment, milk industry and service laboratories. A large variety of PTs are organised. ILVO-T&V is accredited for the PT organisation for the PTs for milk payment laboratories.

2.2.2.3 SWISS NRL

Thomas BERGER, ALP (CH-NRL), presented the interlaboratory trials organised to characterize ALP Somatic Cell Count Standard (SCCS). These SCCS trials, organised at international level, are also used also as PT trials.

The EURL (Véronique DEPERROIS, Head of Unit EDB) reminded that the NRLs have to organize PT trials in their respective countries to verify that the network of laboratories implements correctly the reference methods for total flora and for somatic cell count.

3 MILK HYGIENE

3.1 INTRODUCTION

V. DEPERROIS introduced the work programme of the EURL activities in the milk hygiene field.

3.2 DETERMINATION OF TOTAL FLORA BY ALTERNATIVE METHODS (1/2)

3.2.1 IMPACT OF FACTORS INFLUENCING THE CONVERSION FACTOR IN GOAT'S MILK

Rabeb MILED, Unit EDB, EURL MMP, presented the experimental design and the statistical analysis to be performed by 2012.

Some NRLs suggested to take care of the high SCC levels in goat milk which may affect the robustness of the method.

3.2.2 HARMONISING CONVERSION FACTORS AT EU LEVEL

Laurent GUILLIER, Unit MOB, EURL MMP, presented how the harmonisation of the conversion lines at European level could be envisaged, according to 3 main approaches: one comparing the regression lines, one using a generalized linear model and a last one using a linear mixed effect model. This last option would require the collection of raw data but would enable to determine the impact of the country of origin.

➔ The EURL would settle in 2011 a working group with interested NRLs to investigate the possible harmonization of conversion factors at European level.

3.3 PROFICIENCY TRIALS

3.3.1 STUDY OF RAW GOAT'S MILK SAMPLES USED FOR PT TRIALS FOR THE ENUMERATION OF TOTAL FLORA (2010)

R. MILED presented the outcome of this study. Boric acid mixture had been selected as appropriate bacteriostatic agent.

The PT trial for the NRL would be organised in November 2011 (registration in July).

3.3.2 STUDY OF RAW COW'S MILK SAMPLES USED FOR PT TRIALS FOR THE ENUMERATION OF SOMATIC CELLS (2010 AND 2011)

Alexandra CAUQUIL, Unit EDB, EURL MMP, presented the future study. The target was the organisation of the 2012 PT trial dedicated to SCC in raw cow's milk.

3.3.3 PT TRIAL ON SOMATIC CELLS COUNT

A. CAUQUIL presented the results of the 2010 PT trial on SCC.

28 NRLs had registered for this session, and the results of 25 laboratories could be taken into account for the statistical analysis. 4 laboratories showed unsatisfactory results (lack of repeatability for 2 laboratories and 2 laboratories obtained unsatisfactory z-score). The global performance of the network of NRLs, both in terms of repeatability (RSDr) and reproducibility (RSDr), was good and improved, compared to the former 2008 PT trial on SCC.

3.4 VALIDATION OF ALTERNATIVE METHODS

3.4.1 PROPOSAL FOR A SUMMARY OF VALIDATION CRITERIA

V. DEPERROIS presented a proposal of criteria for the validation of alternative methods for the determination of total flora.

➔ Comments from NRLs on this proposal, in particular on the acceptability values, were requested by 3 June (see circular e-mail dated 04/05/2011).

3.4.2 THIRD-PARTY VALIDATION OF THE INSTRUMENTAL METHODS

B. LOMBARD gave an update on the process of third-party validation/certification of the main instrumental methods for TF in raw milk (Bactoscan and Bactocount). Both equipment suppliers (Foss and Bentley) had contacted 2 certification bodies, MicroVal and AFNOR Certification, which use EN ISO 16140 as validation protocol, to launch the validation process. The EURL had indicated that it would transmit to the certification bodies the validation criteria, once agreed by the NRLs, for use in the validation studies.

3.4.3 ISO/DIS 16297 (REVISION OF IDF 161A)

V. DEPERROIS presented the draft revision of IDF 161 Standard which was to become a join ISO/IDF (ISO 16297), together with comments. The DIS vote was under way, until 16/07/2011.

➔ NRLs were encouraged to comment on this draft Standard through their respective national standardization bodies.

3.5 DETERMINATION OF TOTAL FLORA BY ALTERNATIVE METHODS (1/2)

3.5.1 GUIDANCE ON THE USE OF ISO 21187 FOR THE ESTABLISHMENT OF CONVERSION CHARACTERISTICS

➔ This work would be launched on the basis of the proposals of Lena HODOŠČEK (SI-NRL).

3.5.2 REVISION OF THE CHECK LIST FOR THE LABORATORY VISIT

➔ The revision of the check-list for conducting visits of laboratories in charge of establishing conversion characteristics between instrumental and reference methods would be launched on the basis of proposals from Jolanta ROLA (Piwet, PL-NRL).

3.5.3 DEVELOPMENT OF A MOLECULAR BIOLOGY TOOL FOR THE IDENTIFICATION OF THE BACTERIAL FLORA OF MILK

R. MILED presented the project to be launched at the EURL MMP (Unit EDB) to develop a molecular biology tool using a new Real Time PCR (Lightcycler 1536, Roche) to identify the bacterial flora of raw milk, to check the influence of raw milk flora on the instrumental counting methods and their relationship with the reference method. The study will consist in the development of this new molecular biology tool, using primers already identified for the discrimination of bacterial species of milk and milk products.

3.6 SOMATIC CELL COUNT

3.6.1 CERTIFIED REFERENCE MATERIALS

B. LOMBARD recalled that the need of certified reference materials (CRMs) on SCC in raw milk had been highlighted several times by the EURL MMP with the network of NRLs MMP, and that support from DG SANCO was need for requesting JRC/IRMM (Geel) to develop such CRMs.

➔ K. KOSTENZER agreed to write a letter to JRC/IRMM to support this need of CRM development.

3.6.2 REFERENCE SYSTEM IDF/ISO/ICAR

Thomas BERGER (CH-NRL) gave an update concerning the reference system for somatic cell counting, conducted by an IDF/ISO/ICAR group. He had already presented this project at the 2009 workshop.

Two questionnaires had been dispatched to the reference material (RM) providers and to the routine laboratories, in particular through the EURL and NRL network. The work was currently focused on (i) the preparation of guidelines for preparation of RM, (ii) assessment of the PT schemes and laboratories. A pilot PT test would be conducted.

For more details on this project, refer to newsletters available online (2nd and 1st).

3.7 COLOSTRUM

R. MILED presented the outcome of the enquiry dispatched to the NRLs on national microbiological criteria. No national criterion had been reported in most of the 23 Member

States (respondents) and for the ones having a criterion the same level had been chosen as for raw milk.

Karin KNAPPSTEIN (DE-NRL) explained that it would be difficult to comply with the same threshold than for raw milk. Moreover the enquiry had been focused on legal limits, but not on TF and SCC levels found in colostrums.

➔ The EURL MMP would launch a new enquiry on TF and SCC levels (or other hygienic/pathogenic bacteria) and antibiotic residues found in raw colostrums for direct human consumption, in parallel with a review of the literature, to be conducted in collaboration with Karin KNAPPSTEIN.

3.8 PROPOSAL OF WORK PROGRAMME

1. Inter-laboratory PT :

    a. PT trial on somatic cells count in raw cow's milk (2012)
    b. PT trial on the enumeration of total flora in raw cow's milk (2013)
    c. Homogeneity and stability study of raw cow's milk samples used for PT trials for somatic cells count (started in 2011)
    d. Homogeneity and stability study of raw cow's milk samples used for PT trials for the enumeration of total flora (2012-2013)

2. Analytical development :

    a. Determination of total flora and somatic cells in raw milk by an instrumental method (started in 2007)
        i. Study of raw cow's milk (total flora and somatic cells)
        ii. Study of raw goat's milk (total flora)
    b. Development of a molecular biology tool (Real Time PCR) for the identification of the bacterial flora of raw milk and dairy products
    c. Enquiry on microbiological levels in colostrums and bibliographic review
    d. Usefulness to use PCA+milk *vs.* PCA for the enumeration of total flora in raw milk, according to the reference method EN ISO 4833 (EURL MMP to contact IDF/SC 5 for supporting data)

3. Training for the NRLs: use of the reference method EN ISO 13366-1 for somatic cells count (2012 and possibly 2013).

4 PASTEURISATION TRACERS

4.1 INTRODUCTION

M. NICOLAS introduced the work area on pasteurisation tracers, presenting the topics of the workshop.

4.2 GOAT MILK

4.2.1 PROFICIENCY TEST AND VALIDATION STUDY

4.2.1.1 HOMOGENEITY AND STABILITY STUDIES

Caroline VIGNAUD, CAT Unit, EURL MMP, presented the studies dedicated to check sample homogeneity and stability during the preliminary tests and also during the PT trial dedicated to the determination of alkaline phosphatase (AP) activity in goat's milk. The preliminary study led to set a mandatory date for analyzing the PT samples (3 days after sample preparation)

4.2.1.2 METHOD VALIDATION AND PROFICIENCY TESTING TRIALS

Anne-Cécile BOITELLE, CAT Unit, EURL MMP, presented the outcome of the inter-laboratory trial on AP in goat milk, conducted in December 2010. 22 NRLs participated to this trial, this level of participation was very satisfactory compared to the PTs of 2005 (14 NRLs) and 2007 (19 NRLs). The results of 17 NRLs were retained for statistical analysis.

The trial was designed for 2 purposes: an interlaboratory method validation study and a proficiency testing trial. The results were used to estimate the method repeatability and reproducibility; the results obtained would be proposed to IDF/ISO for inclusion in the revised IDF/ISO Standard.

The results of the proficiency test were also satisfactory: only 3 NRLs obtained results with an action signal (z-scores>3,0).

4.2.2 FIXATION OF AP LIMITS IN GOAT MILK, EUROPEAN STUDY

M. NICOLAS presented this topic. Experiments conducted over several years by EURL and NRLs to investigate a possible legal limit for goat milk showed that a majority of the MSs (15) could comply with the 350 mU/l limit already settled for cow milk but 2 MSs could not comply (Romania and Cyprus because of specific goat species).

➔ The risk managers (DG SANCO with MS Competent Authorities -CAs) should now take a decision. K. KOSTENZER agreed that the preferred option would be to settle a limit of 350 mU/l, with derogation for RO and CY. He needed a justification with a written report of these trials to consult CAs in the working group on food hygiene.

4.3 CHEESE

4.3.1 PROFICIENCY TEST (QUALITATIVE)

C. VIGNAUD presented the outcome of this trial. Upon request of DG SANCO, the EURL has been working on the establishment of a legal limit in cheeses made from pasteurised cow milk. Following the training session in March 2010, a PT was organised to assess whether the NRLs could participate to the study at their national level.

The results of this proficiency test were very satisfactory for the majority of NRLs, for the others, the EURL organized a second session and only 2 NRLs still needed further improvement.

➔ The EURL would dispatch a circular letter to launch the national investigations, together with a form to register all information needed regarding the samples. The NRLs not having participated to the trial could not contribute to this study. The type and number of samples to be analysed would be left for choice to the NRLs provided that they are representative of the national production.

4.3.2 INPUT FROM NRLS, WORK DONE ON NATIONAL INITIATIVE

The EURL, based on its expertise and experience, proposed a tentative limit of 10mU/g to distinguish cheese made from pasteurised cow milk from cheeses made from raw, thermised or microfiltered milk.

Blue veined cheeses were excluded from the study because the method is not applicable to this type of cheese; in fact the mould within blue cheeses also produces AP whilst the protocol used cannot make a distinction between endogenous milk AP and the one produced by the mould.

Some MSs had already performed some experiments on cheeses produced in their MSs.

4.3.2.1 SWITZERLAND

Charlotte EGGER (ALP, CH-NRL) presented the preliminary results obtained in Switzerland. Soft and hard cheeses complied with the tentative 10mU/g limit except for one type of cheese. Indeed one washed rind cheese gave results higher than the proposed limit..

4.3.2.2 OTHER MS

Marina NICOLAS presented the results of the Spanish, Italian and French NRLs.

A limited number of cheese samples (soft cheeses, semi hard cheeses and fresh cheeses) were tested in Spain and showed results well below the tentative limit of 10 mU/g.

Tests performed in Italy corresponded to a particular case, being focused on mozzarella. Due to the stretching process in hot water, all results for cheeses made from raw, thermized and

pasteurised milk were lower than the tentative limit, suggesting that AP determination may not be a pertinent tracer for mozzarella.

Similar results to the Swiss ones were found by the FR NRL: hard, semi hard and soft (Camembert type) cheeses gave results below the tentative limit but 2 types of soft washed rind cheeses (Maroille and Munster) gave results above the tentative limit.

From these preliminary studies, it seems that most tested cheese samples comply with the tentative limit of 10 mU/g. Nevertheless, EURL and NRLs need to tackle the issue of soft washed rind cheeses.

4.4 STATE OF THE ART AT THE INTERNATIONAL LEVEL

4.4.1 EXPERTISE

4.4.1.1 STANDARDIZATION

The repeatability and reproducibility values for goat milk, obtained from the 2010 proficiency testing trial, were incorporated in the draft Standard method (EN ISO 11816-1/IDF 155-1).

A positive vote was casted on the draft revision of EN ISO 11816-2/FIL 155-2 dedicated to AP determination in cheese.

4.4.1.2 COLLECTION OF AP DATA IN CHEESE FROM PASTEURIZED MILK

EURL has submitted at the international level (IDF) a project on collection of AP data in pasteurized cheeses.

4.4.1.3 EC/DG SANCO AND US/FDA APPROACHES ON AP METHODS AND LIMITS IN CHEESE

At the meeting of 8 July 2010 between DG SANCO and FDA/USDA, to which the EURL took part (M. NICOLAS & B. LOMBARD) on equivalence measures related to the hygiene of milk and milk products, US did not accept to discuss the point on harmonization of the official method to use and regulatory limits related to AP content in pasteurised cheese.

> ➔ 1/ Further to this meeting, DG SANCO confirmed its intention to progress with the establishment of an EU regulatory limit for AP in cow milk pasteurised cheese, based on the fluorimetric method.
> 2/ DG SANCO (K. Kostenzer) would ask MS competent authorities to report on any case where cheeses would have been rejected by US at export on the basis AP tests with the colorimetric method.

4.4.1.4 OTHER TRACERS

Work on other tracers is under progress within international expert groups. Currently analytical development is focused on gamma-GT.

4.4.1.5 AP REACTIVATION

It appeared that work needs to be done on the development of a new analytical approach on AP reactivation; the AOAC procedure has weak points and its interpretation is questionable.

4.4.2 MISCELLANEOUS

4.4.2.1 CAMEL MILK

At the FDA Milk Conference (April 2011), the CVRL (Dubaï) made a presentation on pasteurisation tracers in camel milk: contrary to previous publications, it selected LPO versus gamma-GT as heat treatment tracer.

➔ The EURL reminded that it should be kept informed of progress on this topic by CVRL, which is not the case presently..

4.4.2.2 OTHER ITEMS

Bernadette HICKEY (IE NRL) asked if the AP activity had to be tested immediately after pasteurisation. Marina NICOLAS explained that the AP stability in pasteurized milk had been proven in the laboratory and reported in the international literature (no AP reactivation). Therefore B. HICKEY wondered if the request in EC Regulation 853/2004 to test AP immediately after pasteurization could be modified.

➔ K. Kostenzer would further investigate this question with the EURL, possibly distinguishing the own checks (scope of Regulation 853/2004), where AP can be tested immediately after pasteurization, from official controls (scope of Regulation 854/2004) where such immediate testing is not possible.

4.5 AKLALINE PHOSPHATASE WORK PROGRAMME

- AP in cheeses made from pasteurized milk: coordination of national investigations (2011/2012);
- AP in cheeses: validation study/PT trial (2012);
- ISO/IDF standardization works on AP:
  - Revision of Standard EN ISO 11816/IDF 155 parts 1 and 2;
  - project on collection of AP data in pasteurized cheeses;
- Other tracers of heat treatment: Follow up of works conducted at international level;
- Camel milk: follow up of the CVRL study;
- Regulatory requirement for AP determination immediately after pasteurisation: investigation with DG-Sanco.

5 CLOSURE

B. LOMBARD closed the meeting on Tuesday at 4:00 pm, hoping that it met the NRL expectations. He thanked all the attendees for their participation and active contributions to

the workshop. He also informed that next workshop in 2012 would be dedicated to AP activity.