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EU COMMUNITY REFERENCE LABORATORY FOR MILK AND MILK PRODUCTS

Report of the 12th Workshop of the National Reference Laboratories for Milk and Milk Products

(Version 27th July 2009)

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AFSSA LERQAP, Maisons-Alfort, France

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RÉPUBLIQUE FRANÇAISE

1. Opening : Thursday 9th October, 9.30 am

Laurent LALOUX, Head of the Community Reference Laboratory for *Milk and Milk Products* (CRL MMP) at the AFSSA Laboratory for Study & Research on Food Quality & Food Processes (LERQAP), opened the meeting and welcomed the participants (45). Unfortunately he transmitted the excuses from Paolo CARICATO, European Commission, DG SANCO "Health & Consumer Protection", in charge of the EU CRL MMP; Paolo CARICATO took the follow-up of Thierry CHALUS at mid-2008. Then, L. LALOUX presented the French Agency for Food Safety (AFSSA) and particularly the LERQAP activities.

Bertrand LOMBARD, Co-ordinator of the CRL MMP, introduced the meeting that he would chair. He took the opportunity to thank again the Austrian Agency for Health and Food Safety (Austrian NRL) and more particularly Claudia KRALIK for having hosted the previous workshop (11th) dedicated to Alkaline Phosphatase (AP) activity on 9 & 10 October 2008.

Roll-call of delegates

Each delegate introduced itself (see the list of attendance, in annex). 31 NRLs from 26 EU Member States (MSs) and 2 countries (CH, NO) from the European Free Trade Association (EFTA) were represented, as well as the CRL MMP team. Only Sweden was not represented because the appointed laboratory is only working with microbiological methods. The area of responsibility for CRL/NRLs MMP had been largely reduced from year 2006: except the total bacteria count, the scope includes currently chemical and microscopic methods.

The agenda and presentations are available on the following URL:

http://crl.lerqap.free.fr/espace/?key=992b35cfb4758b7243d3deb485bd1371

2. General information

2.1. Tasks and duties of the NRLs

Adrien ASSÉRÉ, Deputy Coordinator of the CRL MMP, presented the slides prepared by P. CARICATO. This is a reminder on the mandate of the NRLs. See the slides.

2.2. Accreditation for NRLs & Official Controls

A. ASSÉRÉ gave a short reminder on the accreditation requirements (see his slides). Indeed, referring to the Regulations EC 2076/2005 (Articles 1 and 18) and 882/2004 (Articles 12 and 33), all the laboratories designated to perform official controls and *a fortiori* CRL and NRLs must be accredited by the end of 2009 for the reference testing methods for raw milk and heat-treated milk prescribed by the Regulation EC 1664/2006 amending Regulation EC 2074/2005:

- EN ISO 4833 for the plate count at 30°C;
- EN ISO 13366-1 for the somatic cell count (SCC);
- EN ISO 11816-1 for the alkaline phosphatase activity.

It was mentioned that the accreditation cannot be based on the draft revisions of these standards.
Some NRLs pointed out that it would be difficult for them to meet this requirement for SCC because they are not performing regularly the microscopic method.

2.3. Various general questions

a. Scope of CRL/NRLs milk and milk products

L. LALOUX proposed to have an open informal discussion about the possible need of enlargement of the current restricted scope of the CRL/NRLs Milk and milk products, based on requests of expertise/information on dairy hygiene at national level.

After a round table, a consensus on 2 types of need arose:

- Better communication between the CRL/NRLs MMP and other CRLs dealing with a biological hazard or chemical contaminant which can be found in milk & MP. For example: *Brucella*, STEC, *Campylobacter*, *Salmonella*,...
- Extension of the mandate to deal with bacteria in milk & MP, included in EC Regulation 2073/2005 and not covered by other CRLs/NRLs, namely: *Enterobacteriaceae*, *Cronobacter (*ex *Enterobacter) sakazakii* and *Bacillus cereus*.

3. Milk hygiene and microbiology

3.1. Total flora (TF)

a. 2007 proficiency testing (PT) trial

Alexandra CAUQUIL, CRL MMP – Unit HMPA, presented the outcome of the PT trial organised in 2007 on TF enumeration. In this trial, most (82%) of the participants shown a satisfactory individual performance, in term of precision (k-ratio or trueness (z-scores). The global performance of the network was good either in repeatability (RSD_r = 1%) or in reproducibility (RSD_R = 4%).

Discussion took place about the requirement of the Standard method EN ISO 4833 to add skimmed milk powder (SMP) in the PCA agar when analysing milk & MP. Some considered that it was not necessary, whereas Veronique DEPERROIS (CRL MMP-Unit HMPA) considered the contrary and Veronique NINANE (BE-NRL) indicated that this was based on trials conducted in the past by IDF.

> ➔ The CRL would ask IDF the outcome of the trials having supported the addition of SMP in the PCA agar for the analysis of MMP and would dispatch this information to the NRLs.

It was commented that the possibility in the test report to use another method than the official reference method may be misleading.

➔ The test reports for the PT trials would not include anymore the possibility to use another method than the official reference method.

b. Study of raw milk samples for PT trial

A. CAUQUIL presented the homogeneity and stability studies performed prior to launch the next 2009 PT trial on TF enumeration reference method.

➔ Upon proposal of Karl ECKNER (NO-NRL), the CRL would consider sending the samples to itself to check the influence of transportation conditions.

c. 2009 PT trial

V. DEPERROIS gave some elements about the organization of the 2009 PT trial dedicated to the TF enumeration.

d. Study on conversion relationship between the reference method and the Bactocount method

V. DEPERROIS presented this study. See her slides. This project started in April 2007 and is still on-going: regular analysis of raw milk samples and data exploitation to study the factors influencing the conversion relationship between the reference method and the Bactocount method.

Some NRLs commented on certain aspects of this study.

➔ The CRL would carry on this study in 2009 and 2010, in collaboration with the DE- & IRL NRLs.

➔ Upon suggestion of Karin KNAPPSTEIN (D-NRL), the CRL would collect information from NRLs on validation of instrumental methods according to EN ISO 16140, as to prepare the 2010 targeted workshop.

e. Analysis of colostrums

A. CAUQUIL presented this topic. The poor information available on TF in colostrum led to design an experimental study in order to estimate acceptable TF level. Contact have been established with an experimental farm of the National Veterinary School of Maisons-Alfort to get samples. The experimental design was also presented. The CRL would test the impact of freezing, as to possibly allow freezing to store samples.

Some NRLs gave advice on the experimental design.

f. Check-list for the laboratory visits on conversion characteristics by NRLs

B. LOMBARD gave information on the way the check-list had been drafted (see CL 2009/04).

➔ This check-list should be now implemented at least for one visit by each NRL before the 2010 targeted workshop.

g. 2008 questionnaire of TF in raw milk

A. ASSÉRÉ presented an update of the enquiry on the determination of total bacterial count in raw milk. The aim was to have a good picture of the situation for the 2010 targeted workshop. But data from 7 MSs were still lacking, and 3 NRLs had to update the data from their MS. It is important that each NRL contact their Competent Authorities and the milk control laboratories to get the actual data.

➔ It was decided to complete the enquiry by the end of 2009 in order to collect the data for all the NRLs.

h. Update on ISO/IDF works – revision of Standard IDF 161

B. LOMBARD presented an update on ISO/IDF work including the revision of the Standard IDF 161 which had been dispatched to the NRLs (see CL 2009/05).

➔ Comments from the NRLs should be sent to B. LOMBARD who would forward them to the relevant IDF/ISO JAT.

3.2. Somatic cell count (SCC)

a. 2008 PT trial

A. CAUQUIL presented the outcome of the PT trial organised last year by the CRL. See her slides. Results were satisfactory (79% NRLs obtained satisfactory individual results) and the performance of the network was clearly improved compared to the former 2002 PT trial.

➔ The PT trial report should be added with the results of the slides.
➔ Given the lack of PT trials organized on SCC with the reference microscopic method and the importance of technician proficiency, the CRL agreed that only in this case, several results could be reported per NRL, in order to be able to test the ability of several technicians in the same trial.

b. Questionnaire on reference materials

B. LOMBARD took the floor to present the outcome of the enquiry on SCC reference materials (see CL 2008/01). Reply from CECALAIT was missing, it should be obtained.

➔ Further action, in particular experimental comparison by the CRL of the different types of reference materials, should wait for further progress of the broader IDF/ICAR project on reference system (see d).

The high need of certified reference materials (CRMs) for SCC in raw milk was reassessed, given the deficiency of the reference method (lack of reproducibility) and its limited use in practice.

➔ The CRL would investigate with P. CARICATO how to approach JRC/IRMM-Geel who could develop such CRMs.

c. SCC by Bactocount method

A. CAUQUIL presented the progress of an on-going CRL project to investigate the conversion relationship between the Bactocount and the reference method. See her slides.

d. Development of reference systems (ICAR/IDF WG)

Thomas BERGER (CH-NRL) presented the work led in the ICAR/IDF working group on reference systems for SCC. See his slides.

B. LOMBARD, who takes part to this group, emphasized the interest of this work for the CRL/NRLs, in order to improve the quality of own checks and official controls on SCC in raw milk, given the deficiency of the microscopic reference method and its limited use in practice.

3.3. Work programme 2010/11

Based on suggestions from Véronique DEPERROIS, the following items were retained for the 2010 tentative work programme (see her slides):

- PT trial on SCC in raw cow's milk (with possibility to report several results per NRL and to enlarge the trial to laboratories in charge at national level to calibrate the instrumental methods);
- Enquiry on PT providers for SCC using the reference method;
- Study of sample types used for PT trials on total flora in raw goat's milk;
- Study of samples types used for PT trials on SCC in raw cow's milk;
- Study of Bactocount for TF and SCC (cont.);
- Study of TF in colostrum (to be reconsidered with P. CARICATO);
- Coordination of NRLs on TF by instrumental methods: 1 enquiry to be completed and 1 enquiry to be launched.
- Contribution to the development of Certified Reference Materials for SCC;
- Follow-up of IDF/ISO/ICAR works.

It was confirmed that the 2010 workshop would be dedicated to alternative methods for TF, to be held after summer. B. LOMBARD suggested that this targeted workshop be held at one NRL location, at its invitation K. KNAPPSTEIN proposed the workshop to be held in Kiel. B. LOMBARD thanked her very much for her kind invitation to come back to Kiel.

4. Alkaline phosphatase activity

Marina NICOLAS (CRL MMP, CAT Unit - AP Team) introduced the AP topic and the targeted workshop organised on 9-10/10/2008 in AGES (Vienna, AT).

4.1. Proficiency tests

Anne-Cécile BOITELLE (CRL MMP, CAT Unit-AP Team) presented the outcome of the 2007 PT Trial and the organisation of the 2009 PT. Then Caroline VIGNAUD (CRL MMP, CAT Unit-AP Team) took the floor to explain how the homogeneity and the stability would be studied. See their slides.

Since the proficiency test comprised a sufficient number of laboratories and samples to comply with the specifications of ISO 5725, parts 1 and 2, the study was considered and evaluated as an international validation trial to generate repeatability and reproducibility figures.

The newly obtained r and R values were better than those defined in ISO 11816-1 | IDF 155-1: 2006, consequently the relevant IDF/ISO JAT has decided to include those values in the Standard EN ISO 11816-1 to characterize the precision characteristics of the Standard method.

4.2. Cheese

Marina NICOLAS presented the analytical tools to measure AP in cheeses. The main optimisation step regarded the extraction. Use of the specific cheese buffer gave better results than milk (the latter is prescribed in the Standard method EN ISO 11816-2), furthermore a special price has been recently negotiated by the CRL for the network of the NRLs.

➔ The CRL is responsible of the revision of the present Standard. Once available, the CRL will distribute to the NRLs the revision draft of EN ISO 11816-2.

Caroline DESBOURDES (CRL MMP, CAT Unit-AP Team) presented the data already collected a large variety on French soft cheeses and some hard cheeses. Luisa PELLEGRINO (IT-NRL) presented an important number of data on Italian pasteurised cheeses. A limit of 6 mU/g for soft and hard cheeses made with pasteurised milk, added of the measurement uncertainty, seemed appropriate for FR and IT. But evidently, prior to the establishment of a European limit, it is important that NRLs from other countries perform tests of cheeses in their respective MSs.

➔ A 2-step approach was agreed:
1/ The CRL will organize a test on the capacity of NRLs to determine the AP activity in cheese and to conclude correctly regarding the pasteurisation or not of the milk used to produce the cheese analysed (call for participation and protocol to be dispatched to the NRLs)[1].

[1] After the workshop, it was agreed with P. CARICATO that this trial would be conducted at the beginning of 2010, and not at the autumn 2009.

2/ Trials should be conducted by the NRLs in their respective countries by March 2011, so as to discuss the outcome during the 2011 general workshop. The aim is to set a limit allowing for the distinction between cheeses made from pasteurised milk and cheeses made with non pasteurised milk.

4.3. Milk from other species than cow

a. Goat's milk

M. NICOLAS presented the work undertaken on goat's milk in several MSs. The issue was that from the experimental data collected, 2 groups of countries were obtained: one complying with the 350mU/l limit for pasteurised milk, and another one (CY, RO) with much higher values.

➔ It was decided to get additional data by the end of 2009, especially from countries not having participated to former trials. A circular letter will be dispatched to launch this last trial before finalizing this topic.

b. Camel's milk

M. NICOLAS reported the work which had been undertaken in collaboration with the Dubai Central Veterinary Research Laboratory (CVRL). The CRL had shown that AP was not a pertinent marker of pasteurisation for camel's milk. The CRL is not anymore the technical partner of CVRL but will be advised regularly on progress of the work and results obtained would be scrutinized by the CRL before they are adopted.

4.4. Alternative methods

M. NICOLAS presented this topic. See her slides. The question was to assess the equivalence of the chemiluminescent EPAS method (ISO 22160) with the fluorimetric reference method ISO 11816-1. A difference in slope of calibration curves had been observed. The CRL has been investigating requirements for method acceptance, and the possible use of the approach of accuracy profile (acceptability limits) to assess the equivalence between 2 methods. Then the CRL would put into practice this theoretical approach to evaluate the chemiluminescent method versus the official fluorimetric method. Comparison would deal with cow's milk (whole, skim and semi-skim) and goat's whole milk.

4.5. AP reactivation

C. VIGNAUD introduced the subject. Samira TABTI, Master student in the CAT-AP Team, presented the project under way on AP reactivation. AP reactivation was shown in cream but there was no reactivation in milk samples. The interpretation of the reactivation test (AOAC) led us to the conclusion that reactivated AP is time dependent. suggesting that. This might not be pertinent.

4.6. Work programme 2010/11

Based on suggestions from M. NICOLAS, the following items were retained for the 2010 tentative work programme (see her slides):

- PT trial: AP in ewe's milk, including a homogeneity & stability study; AP
- Troubleshoot list on Fluorophos equipment and reagents;
- AP in goat's milk: summary of results and recommendation for a legal limit;
- AP in ewe's milk: continuation of the study;
- AP in camel's milk: follow-up of the Dubai study;
- AP in cheeses: test of NRL capacity and coordination of analyses by NRLs;
- Comparison fluorimetric/chemiluminescent method: experimental study;
- AP reactivation: continuation of the follow-up of the issue.

5. Setting up the work programme for the following years

According to the needs identified in the former items of the agenda, and given the resources available in the CRL MMP, a tentative work programme for 2010 and 2011 (see Annex 1) was defined after the workshop at a meeting in Maisons-Alfort on 1st July 2009 with Paolo CARICATO, DG-SANCO.

6. Closure of the workshop

B. LOMBARD closed the meeting on Friday at 2:00 pm, hoping that it met the NRL expectations. He thanked all the attendees for their participation and active contributions to the workshop.

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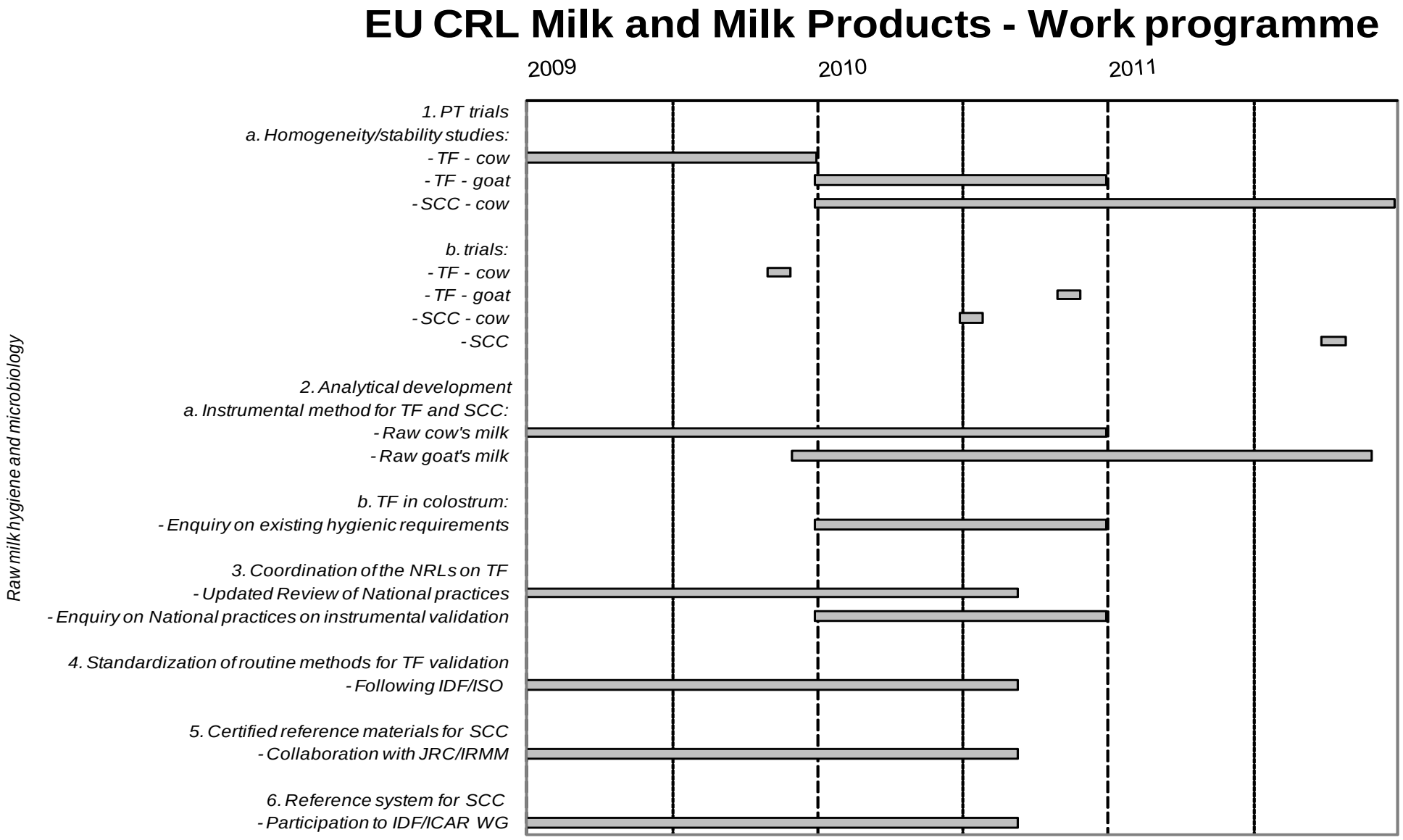
7. Visit of the laboratory

B. LOMBARD guided one group to a visit of the CRL premises.

8. Annex 1


EU CRL Milk and Milk Products - Work programme
2009
2010
2011
Raw milk hygiene and microbiology
1. PT trials
a. Homogeneity/stability studies:
- TF - cow
- TF - goat
- SCC - cow
b. trials:
- TF - cow
- TF - goat
- SCC - cow
- SCC
2. Analytical development
a. Instrumental method for TF and SCC:
- Raw cow's milk
- Raw goat's milk
b. TF in colostrum:
- Enquiry on existing hygienic requirements
3. Coordination of the NRLs on TF
- Updated Review of National practices
- Enquiry on National practices on instrumental validation
4. Standardization of routine methods for TF validation
- Following IDF/ISO
5. Certified reference materials for SCC
- Collaboration with JRC/IRMM
6. Reference system for SCC
- Participation to IDF/ICAR WG

EU CRL Milk and Milk Products - Work programme (continuation)