

Report of the 13<sup>th</sup> Workshop of  
the National Reference Laboratories  
for Milk and Milk Products  
dedicated to total flora in raw milk,  
Maisons-Alfort, 30<sup>th</sup> September & 1<sup>st</sup> October 2010

Version 1 – 30<sup>st</sup> November 2010



## OPENING : THURSDAY 30<sup>TH</sup> OCTOBER, 9.30 AM

Laurent LALOUX, Head of the European Union Reference Laboratory for Milk and Milk Products (EU-RL MMP) at the Anses - Maisons-Alfort Laboratory for Food Safety, opened the meeting and welcomed the participants (42).

L. LALOUX welcomed Paolo CARICATO, DVM, from Unit E2 Food Hygiene of DG SANCO “Health & Consumers”, in charge of the follow-up of the EU-RL MMP.

L. LALOUX gave some news (see his slides) about the change of name of EU-RLs, named European Union Reference Laboratories (EU-RL) from 1<sup>st</sup> January 2010, the EU-RL evaluation launched by DG SANCO and the merging of Afssa with Afsset, in charge of environmental and occupational health risks, giving a new agency Anses (French agency for food, environmental and occupational health safety).

Bertrand LOMBARD, EU-RL MMP Co-ordinator, introduced the meeting. He was glad that at least one NRL representative from all EU Member States (MSs), except Bulgaria, took part to the workshop.

### Roll-call of delegates

Each delegate introduced itself (see the list of attendance, appended): 37 participants from 29 NRLs from 25 EU Members States (MSs) and from Norway and Switzerland. The NRLs from Austria, Slovakia and Sweden were not represented.

Manufacturers of instrumental methods’ equipments, Berte ASMUSSEN (FOSS, DK) and Pierre BROUTIN (Bentley, FR), had been invited on Friday morning in order to present their apparatus. Harrie VAN DER BIJGAART (QLIP, NL) had been also invited on Friday morning to present an update on the revision of the Standard IDF 196 and also the way to establish the conversion characteristic according to EN ISO 21187.

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

<http://crl.lerqap.free.fr/espace/?key=e924fc15d0c2d6ef6bdb7c2537d1d2d3>

## UPDATE ON EUROPEAN COMMISSION ACTIVITIES

### EVALUATION OF EU-RL

P. CARICATO presented the evaluation of the EU-RLs carried out by Civic Consulting on behalf of DG SANCO. Preliminary results would be available by the end of the year. For the milk and milk products area, DG-SANCO is considering to enlarge the mandate of the EU-RL to other bacteria specific to these products, and to make the EU-RL its contact point for any matter of hygiene related to milk and dairy products.

### LINKS WITH THIRD COUNTRIES

Negotiations are on going with the U.S.A. on equivalence agreement for import/export of dairy products, especially for criteria of total flora, somatic cells and phosphatase for cheeses.

The recent Regulation 605/2010 establishes rules for the importation of dairy products in the EU, including new requirements and certificates. A special topic is the import of camel milk, still under discussion.

### HEAT TREATMENT (PASTEURISATION) DETECTION

This subject is a priority because it is also a problem of animal health at import: a dairy product not properly pasteurized may introduce in the EU animal diseases.

## MICROBIAL FLORA OF RAW MILK

Véronique DEPERROIS, EU-RL MMP, Head of Unit HMPA, gave a general presentation on total flora (TF) in raw milk as a hygienic criterion. See her slides.

## PROFICIENCY TESTING TRIALS ON EN ISO 4833

After a recall of the 2007 trial results, Alexandra CAUQUIL, EU-RL MMP, Unit HMPA, presented the outcome of the 2009 trial on TF in raw milk, where the performance of the network was satisfactory.

P. CARICATO considered it was not acceptable that:

- some NRLs did not apply completely the reference method defined in the EC Regulation 1664/2006 (here EN ISO 4833 for total flora);

- one NRL did not reply to the question raised by the EU-RL as a follow up of non satisfactory results in the PT trial.
  - ➔ P. CARICATO asked the EU-RL to send him a list of such cases. DG SANCO may then refer to the respective national Competent Authorities which may reconsider the nomination of NRLs.

## ALTERNATIVE METHODS

### OVERVIEW OF DIFFERENT ALTERNATIVE METHODS

Rabeb MILED, EU-RL MMP, Unit HMPA, presented an overview of different types of existing methods to quantify TF in raw milk. See her slides.

### MEASURABILITY AND MEASUREMENT OF THE PARAMETER “TBC” IN RAW MILK

Hans-Georg WALTE, MRI (DE-NRL), presented the instrumental methodology (flow cytometry) and its impact on TF quantification. See his slides.

- ➔ During the discussion of both presentations, it was confirmed that the colony-count technique (EN ISO 4833) had to be maintained as the reference method, or anchor method to validate alternative methods.

The validation of an alternative method requires the assessment of equivalence between the alternative method and the reference method: this may reveal difficult since in some cases, the analytical target may be different (i.e. instrumental method targeting dead or semi-alive cells whereas EN ISO 4833 counts only viable cells).

## REFERENCE ACTIVITIES

### SUMMARY OF WORK ON TOTAL FLORA: USE OF ALTERNATIVE METHODS AND ROLE OF THE NRLS

B. LOMBARD presented the outcome of the former workshop dedicated to TF in raw milk (Kiel, 11&12 September 2006), the work undertaken since then and the role of NRLs in this topic. See his slides.

### OUTCOME OF THE QUESTIONNAIRE ON NATIONAL PRACTICES

Adrien ASSERE, EU-RL MMP Deputy Coordinator, presented the outcome of the update of the enquiry on national practices for the control of TF in raw milk. See his slides.

A majority (88%) of laboratories using a flow cytometer establishes conversion characteristic (CC) between the reference method and the instrumental method, according to EN ISO 21187 and a majority are using a unique national CC.

The EU-RL would dispatch a report of this enquiry.

- ➔ 1/ The aim was reminded: to have 1 CC per apparatus and per country.
- 2/ The EU-RL, with the help of a working group of NRLs to be settled and consulting apparatus manufacturers, would investigate the possibility to harmonize at European level one CC per apparatus.

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#### VISITS DONE WITH THE CHECK-LIST IN PL

Jolanta ROLA PIWET (PL-NRL) presented the visits that the PL-NRL conducted in 2009 and 2010 of laboratories establishing CC, using the check-list defined by the EU-RL in collaboration with D & BE-NRLs as well as Harrie VAN DER BIIJGAART. See her slides.

J. ROLA suggested that some parts of the check-list should be revised/clarified.

- ➔ The EU-RL, with the help of a WG to be settled, would revise the current check-list, based on suggestions to be forwarded by J. ROLA.

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#### PROGRESS OF THE EU-RL WORK ON THE IMPACT OF FACTORS INFLUENCING THE CONVERSION CHARACTERISTIC (COW'S AND GOAT'S MILK)

A. CAUQUIL, A. ASSERE, and Rabeb MILED (EU-RL MMP, Unit HMPA) presented the work conducted in the laboratory on the factors influencing CC between reference method and a specific instrumental method, Bactocount (Bentley).

The results obtained with raw cow's milk showed a statistical difference between CC in different conditions of milk production/handling but not really impacting on the value of CC.

The EU-RL would prepare a study report of results already obtained for cow's milk and would go on the study for goat's milk.

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#### THE APPLICATION OF FLOW CYTOMETRY IN DETERMINING THE BACTERIOLOGICAL QUALITY OF RAW SHEEP'S MILK IN SLOVAKIA

Peter ZAJAC, NRLM, SK-NRL had prepared a presentation on that topic but could not unfortunately attend the workshop due to the strikes in certain European countries. Nevertheless his presentation is available online.

Giuseppe BOLZONI, IZSLER, for the IT-NRL, presented an Italian project to harmonize the conversion characteristic in Italy. See his slides.

It was an ambitious project, on 2 years, involving 15 laboratories, with a successful outcome.

## PRESENTATION OF INSTRUMENTAL METHODS

### BACTOSCAN

Berte ASMUSSEN, FOSS (Denmark), presented the flow cytometer, Bactocan, manufactured by her company. See her slides.

In particular, she mentioned that normally, the dyeing agent colours only the living cells, except for a transitional period, when the cells have recently died and are still intact. After disintegration, they cannot be stained. This transitional period is not really a problem in raw milk where live cells are in gross majority compared to dead cells (at the difference of pasteurized or sterilized milk).

### BACTOCOUNT

Pierre BROUTIN, Bentley (France) presented the flow cytometer Bactocount of Bentley. See his slides. He has also made a demo in the separate room.

## STANDARDISATION

### VALIDATION OF QUANTITATIVE ALTERNATIVE METHODS IN FOOD MICROBIOLOGY ACCORDING TO EN ISO 16140

B. LOMBARD presented the validation protocol of alternative methods in food microbiology, according to the Standard EN ISO 16140, as well as the current revision process of this standard. See his slides.

It was recognised that no real complete validation study according to EN ISO 16140 had been performed yet on alternative methods for TF in raw milk.

It was also clarified that it is not part of the normal mandate of reference laboratories (EU-RL/NRLs) to validate commercial methods but it is to be undertaken by the bodies dedicated to the validation/certification of alternative methods in food microbiology.

- ➔ For use in the frame of Regulation 853/2004, alternative methods for TF in raw milk shall be validated within 2 years by appropriate validation/certification bodies implementing EN ISO 16140; that is MicroVal, AFNOR Certification and NordVal (the latter if EN ISO 4833 is taken as the reference method for the validation).

#### REVISION OF IDF 161 AND ESTABLISHMENT OF CONVERSION CHARACTERISTIC ACCORDING TO EN ISO 21187

Harrie VAN DEN BIJGAART, QLIP (NL), in his capacity of project leader for EN ISO 21187 and chair of IDF/ISO group on automated methods, presented the establishment of CC according to EN ISO 21187 and the revision of IDF 161 (future ISO 16297). See his slides.

Discussion showed a need to clarify some aspects of EN ISO 21187.

- ➔ It was decided to create a working group of NRLs to collaborate with the EU-RL to write a practical guide to implement EN ISO 21187, based on proposals from Lena HODOSCEK (SLO-NRL).

## CONCLUSION OF THE WORKSHOP

A summary of the outcome and actions to be undertaken is given below.

#### BACTOCOUNT STUDY AT THE EU-RL MMP

- cow's milk: report to be written on the first part of the study, other factors to be further investigated;
- goat's milk: study to be continued.

#### VALIDATION OF ALTERNATIVE METHODS

- alternative methods need to have the same analytical target than the reference method (EN ISO 4833), that is viable cells of total flora in raw milk.
- summary of validation criteria combining IDF 161 and EN ISO 16140: to be prepared by the EU-RL on the basis of Lena HODOSCEK (SI-NRL) proposals.
- requirement of third-party validation of alternative methods, according to EN ISO 16140 protocol, to be undertaken by appropriate validation/certification bodies, and to be obtained by 2 years.

#### CONVERSION RELATIONSHIP ESTABLISHMENT

- to use EN ISO 21187 for the establishment of CC in all MSs.
- development of a practical guide for implementing EN ISO 21187, by the EU-RL with a WG of NRLs.

- EU-RL with a WG of NRLs to investigate the possibility to harmonise CC at European level.
- EU-RL, with a WG of NRLs, to revise the current check-list for conducting visits of laboratories establishing CCs, and then visits to be conducted by NRLs according to this check-list.

This programme of work would be conducted in the following years, at a place depending on resources available at the EU-RL.

#### CLOSURE

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. He also informed the network that next year workshop will be of general scope at the end of April.

#### VISIT OF THE LABORATORY

B. LOMBARD and V. DEPERROIS guided one group to a visit of the EU-RL premises.