



# **2012 Work Programme of the European Union Reference Laboratory for Milk and Milk Products**

*Version 2 – 17 December 2012*

## INTRODUCTION

The Maisons-Alfort Laboratory for Food Safety of Anses (French agency for food, environmental and occupational health safety) foresees to undertake, as European Union Reference Laboratory for milk & milk products (EURL MMP), the following works in 2012 according in particular to (a) the actions planned at the 14<sup>th</sup> Workshop of the National Reference Laboratories (NRLs) (2&3 May 2011).

These actions are part of the current mandate of the EURL MMP, restricted to the control of raw and heat-treated liquid milk (total flora, somatic cells count, phosphatase activity), as well as cheeses for phosphatase, in the frame of the Regulation 853/2004 *laying down specific hygiene rules for food of animal origin*.

The Annex III, Section IX of Regulation 853/2004 is dedicated to raw milk and dairy products:

- Microbiological criteria on total flora at 30°C and on somatic cells count are fixed:
  - o At the level of raw milk production & collection: for raw cow's milk and raw milk from other species milk (Chapter I, clauses I & III);
  - o At the level of preparing dairy products (Chapter II, clause III-criteria for the use of raw cow's milk for further processing).
- Phosphatase activity:
  - o At the level of raw milk production (Chapter I, clause I.3): a reference is made to a negative phosphatase test to characterize the heat-treatment to be applied to raw cow's or buffalo's milk coming from animals not meeting certain requirements on brucellosis or tuberculosis.
  - o At the level of heat treatment of raw milk or dairy products (Chapter II, clause II): the food business operators shall ensure that the heat-treatment satisfies the requirements of Regulation 852/2004, Annex II, Chapter XI.

The EURL foresees in particular to provide a support to the NRLs for the implementation of:

- the Regulation 853/2004;
- the derived Regulation 2074/2005 (modified by Regulation 1664/2006) defining amongst other the testing methods for raw milk and heat-treated milk to be used by competent authorities and food business operators:
  - o to check compliance with the limits for total flora and somatic cells count laid down in Regulation 853/2004, Annex III/Section IX/Chapter I/Part III,
  - o to ensure appropriate application of a pasteurisation process to dairy products, as referred to in Regulation 853/2004, Annex III/Section IX/Chapter II/Part II.

*NB: in brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2012), either multi-annual (on-going programme on several years).*

## 0 GENERAL ASPECTS

### 0.1 GENERAL COORDINATION (EURL MANAGEMENT TEAM, PAFT DEPARTMENT) (MULTI-ANNUAL)

General coordination of the network of the NRLs (dispatch of circular letters and documents, coordination of the scientific and technical support to NRLs, ...).

Relations with DG SANCO, coordination of the scientific and technical advice to DG SANCO, management of annual contract with DG SANCO (annual budgets and work programmes, annual technical and financial reports).

In-house follow-up of EURL activities, expenses, support to EURL units.

### 0.2 WORKSHOP OF THE NRLS (ANNUAL)

The EURL will organise in 2012 the 15<sup>th</sup> NRLs Workshop dedicated to pasteurisation tracers of milk and dairy products. This workshop, aims in particular:

- to make an update of the work undertaken by the EURL since the last general workshop and the last workshop dedicated to this activity (Vienna, 2008);
- to establish a framework programme for the future.

### 0.3 SCIENTIFIC MONITORING AND COMMUNICATION (MUTI-ANNUAL)

The EURL teams will conduct scientific monitoring in its area of competence, as well as communicate on the works conducted as EURL MMP, disseminate the outcome of works in the international scientific community (drafting of written publications, oral presentations and posters to international symposia).

## 1 HYGIENE OF RAW MILK

**Frame** : The Regulation 2074/2005 modified by Regulation 1664/2006 prescribes the reference method for total flora enumeration at 30°C, Standard EN ISO 4833, and the reference method for somatic cells count, Standard EN ISO 13366-1, as well as conditions for the use of alternative methods.

### 1.1 PROFICIENCY TESTING TRIAL

The inter-laboratory proficiency testing (PT) trials organised by the EURL MMP for the NRLs aim at evaluating the ability of the NRLs to apply satisfactorily the methods for the analyses performed in the frame of official controls, prescribed by Regulation 2074/2005 modified.

#### 1.1.1 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY TRIALS ON TOTAL FLORA IN RAW COW'S MILK (MULTI-ANNUAL)

The EURL MMP (Unit EDB) will launch in 2012 an investigation study on the homogeneity and stability of raw cow's milk samples.

In 2009, the EURL MMP had conducted an investigation study (homogeneity and stability) to find a way, such as the addition of a chemical agent, to stabilize sufficiently the total flora (TF) contamination of raw cow's milk, in order to prepare and dispatch itself the samples used for TF enumeration.

The purpose of this study is to optimize the protocol currently used, initially defined in 2009.

#### 1.1.2 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY TRIALS ON SOMATIC CELLS IN RAW COW'S MILK (MULTI-ANNUAL)

The EURL MMP (Unit EDB) will complete in 2012 the investigation study on homogeneity and stability of raw cow's milk samples started in 2011. The aim is to find how to stabilize sufficiently the somatic cell contamination of raw cow's milk, in order to organize PT trials on the enumeration of somatic cells in raw cow's milk samples.

#### 1.1.3 ENUMERATION OF SOMATIC CELLS IN RAW COW'S MILK

The EURL MMP (Unit EDB) will organize in 2012 an inter-laboratory PT trial on the enumeration of somatic cells in raw cow's milk by the reference method, the Standard EN ISO 13366-1, in order to verify that the NRLs apply correctly the reference method for the enumeration of somatic cells.

## 1.2 ANALYTICAL DEVELOPMENT (MULTI-ANNUAL)

### 1.2.1 DETERMINATION OF TOTAL FLORA AT 30°C AND SOMATIC CELLS IN RAW MILK BY AN INSTRUMENTAL METHOD

The EURL MMP (Unit EDB) will continue in 2012 its experimental study on raw cow's and goat's milks, using a flow cytometer (Bactocount) purchased in 2007, as an alternative method to the reference bacterial total flora count and to the somatic cell count (SCC). This study aims at investigating the questions linked to the correlation of the Bactocount to the reference methods for TF and SCC, especially the different factors influencing, for a same apparatus, the value of the conversion factor (variation in breeds, period of lactation, type of feeding, ...).

For that purpose, batches of raw cow's and goat's milk delivered at regular intervals of time will be analysed in parallel by the reference methods and by the Bactocount for TF and SCC.

To verify that the microbial flora has an impact on the conversion relationship between the reference methods and the Bactocount method, the microbial flora of raw milk samples will be identified by PCR-TTGE/DGGE (PCR-Temporal Temperature Gradient Gel Electrophoresis/Denaturing Gradient Gel Electrophoresis).

### 1.2.2 DEVELOPMENT OF A MOLECULAR BIOLOGY TOOL (LIGHT CYCLER 1536) FOR THE IDENTIFICATION OF THE BACTERIAL FLORA OF MILK AND MILK PRODUCTS

The EURL MMP (Unit EDB) envisages to adapt an innovative molecular biology tool (real-time PCR, LightCycler 1536<sup>®</sup> of Roche) for the identification and quantification of the bacterial flora of milk and milk products. The study will consist in developing this new molecular biology tool, using primers already designed for the discrimination of bacterial species of milk and milk products and tested in former projects of the laboratory.

### 1.2.3 ENQUIRY ON MICROBIOLOGICAL LEVELS IN COLOSTRUMS AND BIBLIOGRAPHIC REVIEW

The EURL MMP (Unit EDB) will launch in 2012 a new enquiry on TF and SCC levels (or other hygienic/pathogenic bacteria), as well as on antibiotic residues found in raw colostrums for direct human consumption. In parallel, a review of the literature on this question will be conducted in collaboration with the DE-NRL.

### 1.2.4 USEFULNESS TO USE PCA+MILK AGAR FOR TOTAL FLORA

As agreed at the 2011 workshop, the EURL MMP (Unit EDB) will investigate, through a bibliographic review and an enquiry to IDF/ISO, the usefulness to add milk to the Plate Count Agar (PCA) for the enumeration of the total flora in milk and dairy products. The purpose is to be able to use "normal" PCA, as for other food products than milk & milk products.

### 1.2.5 DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS ON SCC

Given the deficiencies of the reference microscopic method for SCC in raw milk (lack of reproducibility of EN ISO 13366-1 Standard method) and the limited number of laboratories using it, it is of utmost importance to develop Certified Reference Materials (CRMs) to ensure the reliability of SCC analyses of raw milk in Europe, either using the microscopic reference method or instrumental alternative methods. Such CRMS are not currently available. The need to develop CRMs for SCC was highlighted several times by the network of NRLs MMP.

CRMs are needed to calibrate instrumental methods, mostly used for routine analyses of SCC in raw milk, as to have comparable SCC analyses conducted within each European country and between different European countries.

On 11 May 2012, a meeting has been organised with DG SANCO, EC/JRC/IRMM (Geel, BE) and EURL MMP to investigate the need and the feasibility to produce CRMs on SCC in milk. Another meeting has been held on 24 September 2012 at IRMM with IDF/ISO and EURL MMP to further investigate the needs and feasibility of these CRMs.

### 1.3 COORDINATION ACTIVITIES ON TOTAL FLORA DETERMINATION

The EURL MMP (Unit EDB) will go on its work on harmonization of conversion factors (CF), and will in particular settle a WG with interested NRLs to investigate the possibility of CF harmonization at European level.

The EURL MMP will follow up the launching of validation studies by certification bodies (Afnor Certification, MicroVal) of flow cytometers (Bactoscan & Bactocount) for TF determination in raw milk.

The EURL MMP will follow the revision of IDF 161A on the validation of instrumental methods for TF in raw milk.

### 1.4 TRAINING SESSION FOR THE NRLS

The EURL MMP (Unit EDB) will organize for the NRLs in 2012 a training session on the enumeration of somatic cells in raw cow's milk, with the reference method EN ISO 13366-1.

## 2 DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

**Frame:** The Regulation 1664/2006 defines the reference method for official controls in pasteurised cow milk, Standard EN ISO 11816-1, the legal limit for negativity of the test for alkaline phosphatase (AP) activity in correctly pasteurised cow's milk (350 mU/l) and conditions to use alternative methods.

### 2.1 INTER-LABORATORY TRIALS (ANNUAL)

Items 2.1.1 and 2.1.2 were canceled due to the absence of the technician upon EURL contract (3rd February until 2nd of November 2012, with a return at therapeutic half-time).

In order to maintain a PT trial this year, it has been agreed to replace these actions by a new project: a PT trial & method validation inter-laboratory study on Alkaline Phosphatase determination in cow's milk (see 2.1.3).

#### 2.1.1 PRELIMINARY STUDY

~~Following discussions during the IDF/ISO Analytical Week, in May 2011, the EURL MMP has modified the design of validation and proficiency tests on AP determination in cheese set by EURL and NRLs during the Milk and Milk Products Workshop in May 2011. The target of the modification is to include a preliminary study regarding the impact of sample preparation on the performances of the method.~~

~~The international IDF/ISO joint group of experts (including EURL and a number of NRL representatives) deemed important to evaluate the contribution of the clause on preparation of the test sample on the overall variability of the results obtained with the prescribed protocol, before moving to a full validation study, and thereafter to a proficiency test. Consequently, it was decided to proceed in 2012 to a two-part preliminary validation study, with a limited number of laboratories, both on cheese samples as commercially available and on the same samples prepared by EURL according to the clause on preparation of the test sample of the revised draft standard.~~

~~Homogeneity and stability studies will be carried on both the commercial samples as such and on the latter after the test samples have been prepared by EURL. Soft cheese (surface mould/camembert type and washed rind cheeses), semi-hard cheese and hard cheese will be included in the study.~~

~~Following results of this preliminary study, the joint international IDF/ISO group of experts will decide on the type of samples to submit for the full validation study, integrating homogeneity and stability trials. A large participation from NRLs is expected but international laboratories beyond UE (USA, New Zealand, South America) will also be asked to participate.~~

~~The EURL MMP will submit this modified project to the NRLs and seek their comments.~~

### 2.1.2 INTERLABORATORY STUDY

~~Following results of this preliminary study, the IDF/ISO joint group of experts will decide on the type of samples to submit for the full validation interlaboratory study, integrating homogeneity and stability trials. The EURL would encourage a large participation from NRLs but international laboratories beyond UE (USA, New Zealand, South America) would also be asked to participate.~~

~~Results submitted by NRLs to the validation study would be extracted from the total set of results and evaluated as a PT trial.~~

### 2.1.3 PROFICIENCY TESTING TRIAL ON COW'S MILK

The EURL (Team CAT-AP) will organize a trial on the use of the reference method, EN ISO 11816-1, focused on performance of the NRLs at intermediate level. Participants' attention would be drawn to the need to strictly stick to the reference method.

## 2.2 ANALYTICAL DEVELOPMENT (MULTI-ANNUAL)

### 2.2.1 DETERMINATION OF ALKALINE PHOSPHATASE IN CHEESE

The EURL (Marina NICOLAS) continues acting as Project Leader for IDF/ISO on this topic and progresses the item during all stages within the standardisation scheme (see also 2.1).

### 2.2.2 COORDINATION AND PROVISION OF ASSISTANCE ON THE SURVEYS CONDUCTED BY NRLS ON CHEESES MADE FROM PASTEURIZED COW MILK

The EURL (Team CAT-AP) will draft and circulate a table compiling all aspects and criteria that must be filled by NRLs regarding the samples tested in the frame of the project aiming to fix legal limits for AP activity in cheeses made from pasteurized cow milk.

NRLs are expected to collect data on AP content, within EU Member States, in different types of cheese made from pasteurised cow milk and from cow milk having undergone heat treatments milder than pasteurisation (thermisation) or mechanical treatments (microfiltration), and produced under different technologies and processes.

Data will be collected from both pilot and industrial plants operating under well-defined conditions.

Because cheese products are submitted to trade and undergo, among others, analytical controls related to export and import regulations, and considering that EU Member States export outside of Europe, a similar project, led by the EURL MMP, and broadened to the international level is also underway.



### 2.2.3 STUDY ON THE EQUIVALENCE OF CHEMILUMINESCENT METHOD *VERSUS* FLUORIMETRIC METHOD

Depending on the results obtained in 2011, on cow's whole milk, the EURL MMP (Team CAT-AP) will continue the project on equivalence, based on the accuracy profile, between an alternative method, EN ISO 22160 (chemiluminescent method), versus the official method for EU controls, EN ISO 11816-1 (fluorimetric method).

Evaluation of the 2011 results on cow whole milk may lead to reduce the range of equivalence to a single point, the legal limit (i.e. 350 mU/l), or to pursue with levels ranging from 250 to 450 mU/l. Products to be tested are semi-skim and skim cow milk.

### 2.2.4 HEAT TRACERS OTHER THAN AP, DEVELOPMENT OF ANALYTICAL TOOLS

During the IDF/ISO Analytical Week, May 2011, the joint IDF/ISO group of experts decided to formalize Gamma-Glutamyl Transpeptidase (GGT) activity as one appropriate indicator in species in which ALP does not apply.

George ZIOBRO (FDA, USA) briefly presented an open method for determination of GGT using a microwell fluorimetric method. A detailed draft will be made available to the group of experts for initial first round of edits to be included. A new work item form will then be created and reviewed by the experts.

The EURL expressed willingness to actively contribute to this project.

### 2.2.5 REACTIVATED AND MICROBIAL PHOSPHATASE

Some progress was made on this topic with the consideration of a monoclonal antibody (mab) to bovine AP (developed by a French immunoassay innovations company) as a confirmatory method for reactivation of phosphatase. This approach can also provide a solution to problems encountered, particularly in cheese, with heat-stable microbial AP from the microflora. Some research needs to continue to align the MAB method sensitivity with AP action levels.

The EURL (Marina NICOLAS) is co-project leader of the expert group involved in this issue.

### 3 TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION (MULTI-ANNUAL)

#### 3.1 DG SANCO ACTIVITIES

Upon request of the services of DG SANCO in charge of food hygiene:

- Participation to the bilateral US/UE negotiations on veterinary agreement (dairy hygiene, total flora, somatic cell count and phosphatase activity),
- And any other question which may arise during the year.

#### 3.2 PARTICIPATION TO ISO/IDF STANDARDIZATION WORKS

On behalf of DG SANCO (and official nomination as EC representative to CEN/ISO meetings), participation to:

1. The IDF/ISO works on the analytical methods specific to the analysis of milk and milk products in the mandate of the EURL MMP:
  - somatic cells count: reference and alternative methods,
  - total flora: alternative methods,
  - determination of alkaline phosphatase and other pasteurisation tracers in milk and milk products: reference and alternative methods,
  - statistical & sampling aspects;
2. The 2011 IDF/ISO Analytical Week (Tel Aviv, Israel, June 2012) and the meetings of the groups dealing with the topics mentioned above.