



***EU COMMUNITY REFERENCE  
LABORATORY FOR MILK AND  
MILK PRODUCTS***

*Site de Maisons-Alfort*

LABORATOIRE D'ETUDES ET DE RECHERCHES  
SUR L'HYGIENE ET LA QUALITE DES ALIMENTS

**2009 Programme of Work of the Community Reference  
Laboratory for Milk & Milk Products  
Modification 1**

The AFSSA-LERQAP (Laboratory for Studies & Research on Quality of Foods & on Food Processes) foresees to undertake, as Community Reference Laboratory (CRL) for milk, the following works in 2009 according in particular to (a) the actions planned at the 10<sup>th</sup> Workshop of the National Reference Laboratories (NRLs) (28&29 June 2007), and (b) the work programme defined in Annex I of the Framework Partnership Agreement between EC/DG SANCO and the CRL for the period 2006-2010.

These actions are part of the new role of the CRL, 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 CRL foresees in particular to provide a support to the NRLs for the implementation of:

- the Regulation 853/2004;
- the derived Regulation 1664/2006, recently published, 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 2009) , either multi-annual (on-going programme on several years).*

## 1. Hygiene of raw milk

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

### 1.1 Inter-laboratory proficiency testing for the NRLs (annual)

The inter-laboratory proficiency testing trials organised by the CRL 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 1664/2006.

#### a. *Study of sample types used for inter-laboratory trials on total flora in raw milk*

Up to now, the CRL has relied upon CECALAIT to prepare and dispatch raw milk samples for inter-laboratory trials on total flora (TF) enumeration, since CECALAIT controls sufficiently the TF stability of raw milk during transportation.

The CRL (Unit HMPA) will conduct in 2009 an investigation study (stability and homogeneity) to find a way, such as the addition of a chemical agent, to stabilize sufficiently the TF contamination of raw cow's milk, in order to prepare and dispatch itself the samples used for TF enumeration. It is intended to select a formula adapted to TF; formula which would allow the bacteria to grow on plates after the dilution steps.

#### b. *Enumeration of total flora at 30°C*

The CRL (Unit HMPA) will organize an inter-laboratory trial on the TF enumeration at 30°C of raw cow's milk by the reference method, the Standard EN ISO 4833 (total plate count method).

### 1.2 Analytical development (multi-annual)

#### a. *Determination of total flora at 30°C and somatic cells in raw milk by an instrumental method*

The CRL (Unit HMPA) intends to complete for raw cow's milk its experimental study using a flow cytometer (Bactocount), purchased in 2007, as an alternative method to the bacterial total flora (TF) count and to the somatic cells 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 milk delivered at regular intervals of time will be analysed in parallel by the reference methods and by the Bactocount for TF and SCC.

Moreover, the CRL will launch an experimental study using the Bactocount on raw milk of other species than cow.

To go on the collaboration on this topic with the D-NRL (Kiel), a mission to Kiel is scheduled in 2009.

*b. Determination of total flora at 30°C in cow's colostrum*

**Frame:** DG SANCO intends to add hygienic requirements for colostrum in Regulation 853/2004. The CRL and the NRLs have considered that data on acceptable levels of total flora (TF) in colostrum were not readily available, thus a need of investigation from the CRL.

In 2009, the CRL (Unit HMPA) will complete a bibliographical study on the topic of TF at 30 °C in colostrum and will design an experimental study on this determination in colostrum, including the way to get appropriate samples.

Moreover, the CRL will launch an experimental study using the reference method EN ISO 4833 and the Bactocount, as an alternative method to the bacterial count, in order to investigate the questions linked to the enumeration of TF in colostrum. For that purpose, samples of colostrum's batches will be analysed in parallel by the reference method and by the Bactocount.

### **1.3 Coordination of the NRLs on determination of total flora** (multi-annual)

Since the Standard ISO 21187 on the conversion factors between the routine method and the reference method for TF determination has been recently published, the CRL will go on to supervise the NRLs on how they coordinate the implementation of the Standard by the network of laboratories in charge of routine control of raw milk. In particular, all conversion factors should be recalculated according to the Standard in each Member State and it is intended to have only one conversion factor per country.

In 2009, the CRL will in particular envisage what follow-up to give to the outcome of the updated enquiry on national practices, re-circulated in 2008.

### **1.4 Standardization on validation of routine methods for total flora in raw milk** (multi-annual)

IDF/ISO has launched a revision of the Standard IDF 161 detailing the validation protocol of a routine method against a reference method for the TF determination in raw milk.

The CRL will follow this standardization work and will ensure a liaison with the works undertaken as CRL with the NRLs network.

### **1.5 Development of a reference system for somatic cells count in raw milk** (multi-annual)

IDF/ISO has initiated the setting-up of a reference system for SCC in raw milk, given then deficiencies of the microscopic reference method to provide reference values comparable between different laboratories. It is intended that this reference system, in addition to the reference method, would take account of reference materials and of instrumental methods used in routine. A network of expert laboratories is intended to be settled, to define assigned values associated to reference materials used for calibration of instrumental methods.

The CRL will take part to the IDF/ISO working group which will develop this reference system as to envisage how it could be beneficial to its own works, to implement the requirements of Regulation 853/2004 concerning SCC.

## 2. Determination of alkaline phosphatase activity

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

NOTE: This section has been updated according to the outcome of the NRL workshop dedicated to AP (Vienna, 9&10 October 2008).

### 2.1 Inter-laboratory proficiency testing for the NRLs (annual)

Following the 2007 PT trial on AP, the CRL will organize in 2009 a new PT trial on the use of the reference method.

The samples to be tested will be goat's milk. A preliminary study will assess the stability and homogeneity of the sample types to be used in the PT trial. The statistical model applied to the evaluation of the PT, following the conclusions of the working group on statistics, will also be assessed.

### 2.2 Analytical development (multi-annual)

In 2009, the CRL intends to conduct the following activities. It should be stressed that the following work program is scheduled on the basis of a normal yearly activity, assuming that the renovation of the laboratory where AP works are conducted, started end of 2008, will not present important constraints to the execution of the work in 2009.

The company manufacturing the Fluorophos instruments (Advance Instruments) will provide *in situ* a training course for the CRL staff so as to enable them to ensure themselves part of the maintenance and revision of the Fluorophos instruments of the laboratory.

#### *a. Determination of the phosphatase activity in other than cow's milk*

The CRL will continue the study of AP levels for species other than cow's milk. The purpose of this work is to support DG SANCO in prescribing legal limits of AP activity in milk from different species.

#### Goat's milk

In 2009, the CRL will launch a second round of experiments to have a broader view of AP levels in goat's milk. This study should involve in particular NRLs from countries which had not taken part to the first trial. In addition, NRLs would be asked to provide information on the national market of pasteurised goat's milk.

#### For information : camel milk

At the request of DG SANCO, the CRL will develop a collaboration project with the Central Veterinary Research Laboratory (CVRL) of Dubai to characterize the heat-treatment of camel milk.

Preliminary results of an informal collaboration with CVRL in 2008 tend to show that AP is not a pertinent indicator of pasteurisation of camel's milk.

All costs of the study will be covered by CVRL.

*b. Determination of alkaline phosphatase in cheeses*

In 2008, the CRL has introduced a proposal to revise the international Standard related to the determination of AP activity in cheese, in the framework of the relevant IDF/ISO Joint Action Team. The proposal highlighted the main changes to introduce in the revision. In 2009, the CRL will draft and circulate the revised text and follow-up comments received..

In 2009, the CRL will coordinate further experiments on soft and hard cheeses by NRLs, especially other LNRs than those having already performed tests. For the time being, the experiments should concern soft and hard (not blue) cheeses from cow's milk.

The expected end-product is the fixation of an AP limit allowing for the distinction between cheeses made from pasteurised milk and cheeses made with non pasteurised milk.

*c. Comparison of the chemiluminescent/fluorimetric methods*

The comparison performed in 2008 between the chemiluminescent method (Novalum) and the current regulatory reference method, the fluorimetric method (Fluorophos) showed an improvement but there is still a non-negligible bias between the two methods.

In 2009, on the more general topic of method equivalence for AP determination, the CRL intends to investigate at first requirements for method acceptance, then the possible use of the approach of accuracy profile (acceptability limits) to assess the equivalence between 2 methods.

*d. Reactivated phosphatase*

The CRL will conduct a preliminary study on AP reactivation. The CRL intends to investigate conditions that promote AP reactivation, the specificity of the phenomenon to matrices/processes, comprehension and, if possible, optimisation of the analytical method used to determine reactivated phosphatase.

### **3 Assistance to the NRLs**

#### **3.1 Training courses**

##### *a. Determination of alkaline phosphatase in cheese*

Further to a request at the 2008 workshop, the CRL will organize in 2009 a demonstration session for the NRLs on AP determination in cheese. If needed, training sessions may also be organized. The CRL would thereafter organize a proficiency assessment of the NRLs.

##### *b. Others*

Upon requests of NRLs, the CRL may receive NRL staff for individual training on specific topics.

### **4 NRLs Workshop**

The CRL will organise in 2009 the 12<sup>th</sup> NRLs Workshop, of general scope. In particular, this workshop will enable:

- to make a point of works undertaken by the CRL since the last general Workshop of 2007, in particular further to the 2008 Workshop dedicated to the phosphatase activity;
- to envisage the work programme for the following years.

### **5 Technical and scientific assistance to the European Commission**

#### **5.1 Participation to ISO/IDF standardization works**

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

- the IDF/ISO works on the analytical methods specific to the analysis of raw milk:
  - somatic cells count: reference and alternative methods,
  - total flora: alternative methods,
  - phosphatase test: reference and alternative methods.
- The 2009 *IDF/ISO Analytical Week* and the meeting of the groups dealing with the topics mentioned above.