



***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

**2008 Programme of Work of the Community Reference
Laboratory for Milk & Milk Products**

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 2008 according in particular to (a) the actions planned at the 10th 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 for:
 - o raw cow's milk and for raw milk from other species milk (Chapter I, III),
 - o dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing).
- Phosphatase activity:
 - o In Chapter I, I.3, a reference is made to a negative phosphatase test to characterize the heat-treatment applied to certain raw milks at the primary production stage (Chapter I, I.3).
 - o Chapter II(II) includes requirements on heat-treatment of raw milk or dairy products, applicable to food business operators. A cross reference is made to the general 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 2008) , either multi-annual (on-going programme on several years).

1. Inter-laboratory proficiency testing

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.

1.1 Somatic cells count (annual)

Frame : The Regulation 1664/2006 prescribes the reference method for somatic cells count, Standard ISO 13366-1 as well as conditions for the use of alternative methods.

The CRL (Unit HMPA) will organize an inter-laboratory trial on somatic cells count by the reference method, the Standard ISO 13366-1.

Two CRL staffs will visit CECALAIT for establishing the experimental design of the samples for the trial, to be prepared by CECALAIT.

1.2 Determination of alkaline phosphatase activity (annual)

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

Criteria to evaluate the individual performance of NRLs

At the last workshop of the NRLs Milk (7&8 June 2007), it was agreed that the CRL together with experts of some NRLs would define pertinent criteria to evaluate the individual performance of NRLs participating to PT trials organized by the CRL on AP activity. The outcome would be presented at the 2008 workshop dedicated to AP.

It is intended to organize one meeting of the NRL working group in Brussels, in conjunction with one meeting of the DG AGRI chemical experts' working group.

2 Analytical development

These works are conducted in the CRL laboratory alone.

2.1 Determination of total flora at 30°C in raw milk (multi-annual)

Frame : The Regulation 1664/2006 prescribes the reference method for total flora at 30°C, Standard EN ISO 4833 as well as conditions for the use of alternative methods.

The reference method for evaluating this parameter is the Standard EN ISO 4833 (enumeration of bacteria on Petri dishes). However, this reference method is generally not used in routine analyses for the hygiene control of raw milk. Alternative methods are used instead, mainly instrumental ones based on flow cytometry (such as the Bactoscan or Bactocount apparatus). Their use is allowed in Regulation 1664/2006 under certain conditions.

a. Coordination of the NRLs

Since the Standard ISO 21187 on the conversion factors between the routine method and the reference method 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 2008, the CRL will in particular update the enquiry on national practices by re-circulating the questionnaire to NRLs (update of NRL replies or additional replies). It will also prepare, in collaboration with the D and BE NRLs, a check-list for the NRLs to conduct the visits of laboratories in charge of establishing the conversion factors.

b. Study of the alternative methods

The CRL (Unit HMPA) will go on the experimental studies using a flow cytometer (Bactocount), purchased in 2007, as an alternative method to the bacterial count, in order to investigate the questions linked to the correlation of this method to the reference method, 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 milk delivered at regular intervals of time will be analysed in parallel by the reference method and by the Bactocount.

c. Scientific and technical support

IDF/ISO intends to launch a revision of the Standard IDF 161 detailing the specificities of validation of a routine method against a reference method for the determination of total flora in raw milk. The CRL would follow this standardization work and would ensure a liaison with the works undertaken as CRL with the NRLs network.

2.2 Determination of total flora at 30°C in colostrums (multi-annual)

Frame: DG SANCO intends to add hygienic requirements for colostrums in Regulation 853/2004. But the CRL and NRLs consider that data on levels of total flora in colostrums are not readily available.

In 2008, the CRL (Unit HMPA) will launch a bibliographical study on the topic and will design the experimental study on determination of total flora at 30°C in colostrums, including the way to get appropriate samples, before conducting the study the year after.

2.3 Determination of somatic cell count in raw milk (multi-annual)

Frame : The Regulation 1664/2006 prescribes the reference method for somatic cell count in raw milk, Standard ISO 13366-1 as well as conditions for the use of alternative methods.

The reference method for evaluating this parameter is the Standard ISO 13366-1 (microscopic technique). However, this reference method is generally not used in routine analyses for the hygiene control of raw milk. Alternative methods are used instead, mainly instrumental ones based on flow cytometry (such as the Fossomatic or Bactocount apparatus). Their use is allowed in Regulation 1664/2006 under certain conditions.

The CRL (Unit HMPA) will launch experimental studies using a flow cytometer (Bactocount), purchased in 2007, as an alternative method to the microscopic technique, in order to investigate the questions linked to the correlation of this method to the reference method, in parallel to the study of the use of the same apparatus for total flora (see 2.1.b).

2.4 Determination of alkaline phosphatase activity (multi-annual)

In 2008, the CRL (Unit CALAS) 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. If renovation of the CALAS laboratory is undertaken in 2008, the work may have to be re-scheduled to fit with the constrains of renovation.

One CRL staff will follow a training course by the company manufacturing the Fluorophos instruments, as to be able to ensure itself 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 go on the pasteurisation studies on goat's and ewe's milk, whose purpose is to support EC advising an appropriate legal limit on alkaline phosphatase (AP) activity for ewe's and goat's pasteurised milk.

Goat's milk

For the 2008 NRLs workshop dedicated to AP, the CRL will finalise its proposal of a legal limit for correctly pasteurised goat's milk. The proposal has been postponed in order to study results of Greek goat's milk that might lead to a higher level than the one envisaged by the CRL and NRLs collaborating to this project (from Portugal, Spain, Norway, Romania and Switzerland).

Ewe's milk

The CRL will study thermal inactivation of AP in ewe's milk, with input from collaborating NRLs. The CRL will establish the kinetics of AP inactivation in parallel with decrease of the microbial load of the milk samples in order to propose a legal limit for correctly pasteurised ewe's milk.

Camel's milk

To characterize the heat-treatment of camel's milk, the CRL intends to launch a study in collaboration with the Central Veterinary Research Laboratory (CVRL) of Dubai.

The first phase of the study, to be launched in 2008, would aim at assessing the possibility to use AP activity as an pertinent indicator of pasteurisation of camel's milk.

The specific costs of the entire study is to be covered by a contract with CVRL.

b. Determination of alkaline phosphatase in cheeses

As a first step to the relevant standardisation procedure, the CRL will prepare for IDF/ISO a revised draft Standard related to the determination of AP activity in cheese.

The CRL will continue the study on the residual AP activity in soft cheese and will compile these results with those forwarded by other NRLs. The expected end-product is the fixation of a 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 Spring 2007 between the chemiluminescent method (Novalum) and the current regulatory reference method, the fluorimetric method (Fluorophos) showed a bias between the two methods.

Based on a modified protocol to be proposed by the manufacturer of Novalum, the CRL intends to conduct a new comparison study in 2008

3 Assistance to the NRLs

3.1 Training courses

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

4 NRLs Workshop

The CRL will organise in 2008 the 11th NRLs Workshop, targeted on alkaline phosphatase activity. In particular, this workshop will enable:

- to make an update of all the works undertaken by the CRL on this topic, especially since the former workshop dedicated to this topic (2002);
- to define limits for AP in goat's and possibly in ewe's milk;
- to envisage the feasibility of a criteria approach for AP activity.

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 *IDF/ISO Analytical Week* and the meeting of the groups dealing with the topics mentioned above.