



***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

**2010 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 2010 according in particular to (a) the actions planned at the 12th Workshop of the National Reference Laboratories (NRLs) (28&29 May 2009), 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 current mandate 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 2010) , 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 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 Inter-laboratory proficiency testing for the NRLs

The inter-laboratory proficiency testing (PT) 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 goat's milk*
(multi-annual)

The CRL (Unit HMPA) will conduct in 2010-2011 an investigation study (stability and homogeneity) to find a way, such as the addition of a chemical agent, to stabilize sufficiently the total flora (TF) contamination of raw goat's milk, in order to organize PT trials on TF enumeration in raw goat's milk samples. 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 somatic cells*
(annual)

The CRL (Unit HMPA) will organize in 2010 an inter-laboratory trial on somatic cells count in raw cow's milk by the reference method, the Standard EN ISO 13366-1. CECALAIT will prepare and dispatch the samples for the trial. For this trial, NRLs may recommend the CRL to include laboratory(ies) in charge of calibrating instrumental methods at national level.

In addition, the CRL would enquire on the availability of external PT providers who would/could organize PT trials on SCC in milk by the reference 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 in 2010 its experimental study for raw cow's milk, 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 a similar experimental study using the Bactocount on raw goat's milk.

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

Frame: In the frame of Regulation 853/2004, national hygienic requirements are referred to for colostrums.

In 2010, the CRL will conduct an enquiry to the NRLs to collect existing national hygienic requirements/microbiological criteria on cows' colostrum.

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

Since the publication of the Standard EN ISO 21187 on the conversion factors between the routine method and the reference method for TF determination, the CRL has been supervising 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 2010, in view of preparing the 2010 workshop dedicated to alternative methods for TF in raw milk, the CRL will in particular:

- complete the outcome of the updated enquiry on national practices, re-circulated in 2008;
- launch an enquiry to collect national practices in terms of validation of the instrumental methods according to the Standard EN ISO 16140.

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 certified reference materials for somatic cells count in raw milk
(multi-annual)

Given the deficiencies of the reference microscopic method for SCC in raw milk (lack of reproducibility) and the limited number of laboratories using it, it is of utmost importance to develop Certified Reference Materials, which are not currently available.

In 2010, the CRL intends to bring its support to identify the possibilities to develop certified reference materials by the JRC/IRMM in Geel. For that purpose, DG SANCO is to contact JRC to organize a meeting.

1.6 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 the 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 the calibration of instrumental methods.

The CRL will go its participation to the IDF/ICAR working group in charge of developing this reference system as to envisage how it could be beneficial to the CRL/NRLs 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 EN 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.

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

The CRL (Team CAT-AP) will organize in 2010 a PT trial on the use of the reference fluorimetric method (EN ISO 11816-1) in ewe's milk.

- A preliminary study will assess the stability and homogeneity of the samples to be used in the PT trial.
- If, as expected, the proficiency test comprises a sufficient number of laboratories and samples to comply with the specifications of ISO 5725, parts 1 and 2, the study will also be considered and evaluated as an international validation trial to generate repeatability and reproducibility figures.

2.2 Analytical development (multi-annual)

In 2010, the CRL (Team CAT-AP) intends to conduct the following activities.

a. *Fluorophos reagents and instrument*

Considering the occurrence of repeated problems with the Fluorophos reagents and the instrument, the CRL will establish a troubleshoot list to be sent to the manufacturer. The latter was already informed of the ongoing procedure and is willing to discuss the issues and find solutions to the problems encountered.

This action has been launched in 2009 and will be continued in 2010.

b. *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

Following the experiments launched in the fall 2009, the CRL will compile all results obtained since 2003 so as to support DG SANCO in prescribing legal limits for AP in goat's pasteurised milk.

Ewe's milk

CRL will work on the establishment of the time-temperature conditions needed to inactivate AP in ewe's milk. In fact, because of the high fat content of ewe's milk, the heat load necessary to the pasteurisation of this type of milk is more important than for cow's and goat's milk.

Once the pasteurisation conditions for ewe's milk would have been identified, the CRL intends to start some preliminary work on raw and laboratory pasteurised milk to initiate the project aiming to the setting up of AP limits in ewe's pasteurised milk

Camel's milk

Further to an initial study conducted by the CRL and having shown that AP is not an appropriate heat-treatment marker for camels' milk, the CRL has developed and submitted in 2009 to the Central Veterinary Research Laboratory (CVRL) of Dubai a collaboration project to characterize the heat-treatment of camel's milk. As a consequence of recent discussions, the CRL will not be in the future the technical partner of CVRL in the frame of this collaboration but the CRL is still closely involved in this project at the request of DG SANCO. Practical work will not start before the CRL has indorsed the work programme. The CRL will be advised regularly on progress of the work and results obtained will be scrutinized by the CRL before they are adopted.

c. Determination of alkaline phosphatase in cheeses

In 2009, the CRL has drafted a revised text of the international Standard related to the determination of AP activity in cheese and circulated it for comments to the relevant IDF/ISO Joint Action Team. The CRL will continue acting as Project Leader for this topic and will progress the item during all stages prescribed within the official procedures of standardisation.

The CRL will continue the coordination of experiments on soft and hard cheeses (cow's milk cheese) by NRLs.

Prior to that, the CRL will organize in 2010 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. Three samples of cheese, made from pasteurised, raw and mild heat treated milk, as well as calibration samples, will be sent to the NRLs for analysis.

It is expected that by the next general 2011 workshop, information from most of the NRLs will be available so as to enable setting up of AP limits allowing for the distinction between cheeses made from pasteurised milk and cheeses made with non pasteurised milk.

d. Comparison of the chemiluminescent/fluorimetric methods

In 2009, in the frame of the general topic of method equivalence for AP determination, the CRL would have investigated requirements for method acceptance, and the possible use of the approach of accuracy profile (acceptability limits) to assess the equivalence between 2 methods.

In 2010, the CRL intends to put into practice this theoretical approach to evaluate the chemiluminescent method *versus* the official fluorimetric method. Comparison will deal with cow's milk (whole, skim and semi-skim) and goat's whole milk.

e. *Reactivated phosphatase*

No further experimental work will be conducted in 2010, but the CRL will continue a theoretical exploration of the issue. An exchange of views with scientific experts of the US/FDA competent laboratory is envisaged.

3 Assistance to the NRLs

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

4 NRLs Workshop

The CRL will organise in 2010 the 13th NRLs Workshop, dedicated to the use of alternative methods for total flora in raw milk. It is intended to have this workshop hosted by one NRL, most probably the D-NRL in Kiel.

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 2010 *IDF/ISO Analytical Week* (Montreal, CA, May) and the meetings of the groups dealing with the topics mentioned above.