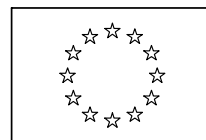




AGENCE FRANÇAISE  
DE SÉCURITÉ SANITAIRE  
DES ALIMENTS

*Site de  
Maisons-Alfort*

LABORATOIRE D'ÉTUDES  
ET DE RECHERCHES SUR  
LA QUALITÉ ALIMENTAIRE  
ET SUR LES PROCÉDÉS  
AGROALIMENTAIRES



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

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# 2007 Technical report of the Community Reference Laboratory for Milk and Milk Products

**10/03/2008**

AVENUE DU GÉNÉRAL DE GAULLE  
F-94706 MAISONS-ALFORT CEDEX  
TÉLÉPHONE : + 33 (0)1 49 77 13 00  
TÉLÉCOPIE : + 33 (0)1 43 68 97 62  
[www.afssa.fr](http://www.afssa.fr)

## Introduction

The AFSSA-LERQAP (Laboratory for Studies & Research on Quality of Foods & on Food Processes) had foreseen to undertake, as Community Reference Laboratory (CRL) for milk & milk products, the following works in 2007 according in particular to the actions planned at the 8<sup>th</sup> Workshop of the National Reference Laboratories (NRLs) (30<sup>th</sup> June-1<sup>st</sup> July 2005), as well as to 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 liquid milk as raw material (total flora, somatic cells count, and phosphatase), in the frame of the Regulation 853/2004 *laying down specific hygiene rules for food of animal origin* (its Section IX is dedicated to raw milk and dairy products). This new regulation is applicable from 1<sup>st</sup> January 2006.

NB: In brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2007), either multi-annual (on-going programme on several years).

## 1. Inter-laboratory proficiency trials

### 1.1. Total flora at 30°C

(annual)

**Frame:** In Section IX of Regulation 853/2004, microbiological criteria have been fixed for raw milk (Chapter I, III) and for dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing). They include criteria on plate count at 30°C for raw cow's milk and from other species.

The Regulation 1664/2006, recently published, defines amongst other the testing methods for raw milk and heat-treated milk to be used by competent authorities and food business operators, including the reference method for total flora at 30°C, Standard EN ISO 4833 as well as conditions for the use of alternative methods.

The CRL (Unit HMPA) has organised in 2007 an inter-laboratory trial on the enumeration of total flora at 30°C by the reference method, the Standard EN ISO 4833 (total plate count method). In accordance with the 2006 Workshop of the NRLs on total bacterial count, the participants were not only the NRLs but also the laboratories in charge in each country to establish the conversion relationship between the reference method and the instrumental methods.

The inter-laboratory proficiency testing has been launched by a circular letter of 23 April 2007. The CRL (Unit HMPA) has prepared and dispatched in advance (Week 38) the instructions and test report for the trials. CECALAIT has prepared the samples (6 samples of raw milk at different level of contamination to total flora) and dispatched them in October (Week 40) to the 48 participating laboratories.

The CRL has collected the results and is currently preparing the report to be soon dispatched.

## **1.2. Determination of alkaline phosphatase activity**

**(annual)**

**Frame:** In Section IX of Regulation 853/2004, a reference to a negative phosphatase test is made to characterize the heat treatment applied to certain raw milks at the primary production stage (Chapter I, I.3).

Chapter II(II) of Section IX is dedicated to 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 Regulation 1664/2006, recently published, includes more detailed requirements for heat treatment, with a reference to the phosphatase test. It also 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.

The CRL has organized a second proficiency test on the use of the reference method (ISO 11816-1 / IDF 155-1:2006). The inter-laboratory proficiency testing has been launched by a circular letter of 10 September 2007. The CRL (Unit CALAS) has prepared and dispatched in advance (Week 46) the instructions and test report for the trials to point out the importance of a strict and total application of the method was highlighted to the participating NRLs.

The samples of fresh milk have been prepared and dispatched in November (Week 48) to the participating laboratories (20 NRLs). Each laboratory received 42 samples of cow's whole, semi-skim and skimmed milk to be submitted to the test.

Laboratories using other methods received the samples at their request, but their results will not be included in the evaluation of performances.

The evaluation report will be soon available.

## **2. Analytical development**

### **2.1. Determination of total flora at 30°C (multi-annual)**

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 apparatus). Their use is allowed in the Regulation 1664/2006.

#### *a. Coordination of the NRLs*

At the 2007 workshop, the CRL has made a stand-point on the implementation of the Standard ISO 21187 (conversion factors between the routine method and the reference method) by the network of laboratories in charge of routine control of raw milk.

### *b. Study of the alternative methods*

The CRL (Unit HMPA) has begun experimental studies using a flow cytometer (Bactocount, Bentley Instruments, purchased at the beginning of the year) 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 were delivered at regular intervals of time and analysed in parallel by the reference method and by the Bactocount. In total, 330 samples analysed in 2007.

In December 2007, the CRL (Adrien Asséré and Alexandra Cauquil) has visited the German NRL, BfEL, located in Kiel for collaborating on that topic.

### *c. Scientific and technical support*

Contrary to what foreseen, the specificities of validation of a routine method against a reference method for the determination of total flora in raw milk will not be incorporated in an annex to EN ISO 16140 under revision, but will be dealt in a specific Standard, IDF 161, to be revised by the IDF/ISO JAT "Routine analysis in quantitative microbiology". The CRL has followed this process and contributed to it.

## **2.2. Somatic cells count (multi-annual)**

**Frame :** In Section IX of Regulation 853/2004, microbiological criteria have been fixed for raw milk (Chapter I, III) and for dairy products (Chapter II, III-criteria for the use of raw cow's milk for further processing). They include criteria on somatic cells count for raw cow's milk and from other species.

The Regulation 1664/2006, recently published, includes the description of testing methods for raw milk and heat-treated milk, including the reference method for somatic cells count, Standard ISO 13366-1 as well as conditions for the use of alternative methods.

At the 2004 Workshop of the NRLs dedicated to the reference microscopic method for somatic cells count (SCC), it was identified that a major aspect to ensure and to enhance the quality of this counting, in addition to the harmonisation and improvement of the reference method, was the availability of qualified reference samples to "calibrate" the analyses.

The CRL has prepared and sent by circular letter dated 03/10/07 a questionnaire to the European organisations known to provide such reference samples to SCC, as to review the condition of their production, QA measures. 7 organizations have replied. The CRL has prepared and dispatched the outcome of this enquiry by circular letter dated 13/02/08. The outcome will be discussed at the next general NRL workshop.

### **2.3. Enumeration of Enterobacteriaceae (multi-annual)**

**Frame:** In the EC Regulation 2073/2005 on microbiological criteria for foods, the criteria of the former Directive 92/46 on coliforms for dairy products have been replaced by criteria on *Enterobacteriaceae*. Several NRLs had expressed their concern about the consequence of this change. In particular in analytical terms: the new horizontal Standard methods for enumeration of *Enterobacteriaceae* (ISO 21528) require a confirmation in two steps, and the performances of media were reported to lack of consistency between manufacturers.

The CRL (Unit HMPA) conducted a study investigating the applicability to milk products of the new horizontal Standard methods, the Standard ISO 21528 in 2 parts (part 1: most probable number technique and part 2: colony-count technique).

The following performance criteria were evaluated:

- Specificity,
- Trueness,
- Limit of detection/enumeration.

The CRL also compared the performance of media from different manufacturers.

The study has been finalised at the beginning of 2007. The CRL has prepared and dispatched by circular letter dated 20 July 2007 the report to the NRLs.

### **2.4. Determination of alkaline phosphatase activity (multi-annual)**

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

- Following the NRL workshop held in June 2007, a new circular letter (sent on 10/07/2007) was addressed to NRLs regarding trials on the phosphatase level in ewe's and goat's milk.  
Norway, Romania, Greece and Cyprus expressed willingness to participate to the study, either by pasteurizing the raw milk samples in their own place, according to the instructions sent, or by shipping the raw milk samples (frozen) to CRL so that we proceed ourselves with the heat treatment.  
Spain, Portugal, Ireland, Romania, Slovakia and Switzerland have already provided samples and/or data.
- CRL has re-activated exchange of information on pasteurised camel milk with the Central Veterinary Research Laboratory in Dubai. CVRL considers this issue as an important and necessary step in their endeavours to export camel milk products to the European Union.

CRL has drafted a proposal on the pertinence of the Alkaline Phosphatase (AP) activity as an indicator of correct pasteurisation of camel's milk. This will serve as the basis of a formal collaboration study between the two partners, to be launched in 2008.

- CRL has also established contact with the CIRAD (Centre de coopération internationale en recherche agronomique pour le développement) on microbiological aspects and heat load tracers related to camel milk.

*b. Determination of alkaline phosphatase in other dairy products than milk*

- To conclude the study conducted on soft cheese from pasteurized cow's milk of French origin, the CRL proposed a tentative limit of 10mU/l for correctly pasteurized soft cheese (cow).

The CRL insisted on the fact that the proposed limit needed to be confirmed with data on same type of cheese from different Member States. Only Ireland replied to the request till end of 2007, providing soft cheese samples to be analysed by the CRL.

- Some NRLs expressed interest for limits in butter and cream.

*c. Criteria approach*

CRL was asked to compare the “official” reference method prescribed in the Regulation 1664/2006, with alternative methods.

The comparison performed by the CRL in spring 2007 between the chemiluminescent method (Novalum) and the fluorimetric method (Fluorophos) on cow's whole, semi-skim and skim milk and on ewe's milk was evaluated in collaboration with Roger Wood, the UK –and international- expert on statistics. It showed a non-negligible bias between the two methods.

The manufacturer of Novalum will propose a modified protocol and the CRL will conduct a new comparison study in 2008.

*d. Determination of alkaline phosphatase in other dairy products than milk*

- It should be mentioned that CRL and NRLs consider it of essential interest to tackle the issue on re-activated phosphatase, in milk products with a high fat content, from a fundamental and from an analytical point of view.

The item will be on the agenda of the NRL Workshop 2008, dedicated to Alkaline Phosphatase.

- CRL has introduced a new work item to ISO and IDF about revision of the international standard on determination of phosphatase activity in cheese. Critical points of the existing analytical procedure were demonstrated and preliminary results obtained with the revised protocol presented.

The Joint IDF/ISO Action Team responded to this new work item proposal positively.

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

### **3.1. Training courses**

A two-days training course on determination of Alkaline Phosphatase in milk, ISO 11816-1/IDF 155-1:2006 has been organised for two staffs of the Cypriot NRL on 3-4 December.

### **3.2. Visit of NRLs**

The CRL has visited the German NRL, BfEL, Kiel (see item 2.1.b).

## **4. NRLs Workshop**

The CRL has organised on 28<sup>th</sup> & 29<sup>th</sup> June 2007 in Maisons-Alfort the 10<sup>th</sup> Workshop of the NRLs, of general scope. It has dealt in particular with:

- works undertaken as the former CRL Milk (enumeration of staphylococci, of *Enterobacteriaceae*, detection of staphylococcal enterotoxins),
- Total flora,
- Somatic cell count,
- Alkaline phosphatase activity.

A Working Group was set up to investigate (i) how to derive  $\sigma_p$  and (ii) how to evaluate participants' performance, in the particular case of AP activity. The WG was convened by the CRL and comprised Roger WOOD, Miguel RODRIGUEZ (SP), T. BERGER (CH), Karl ECKNER (NO) and Jan DE BLOCK (CVUO, BE).

For any further information, please refer to the report (dispatched on 27/08/2007).

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

### **5.1. Participation to Codex/CEN/ISO/IDF meetings**

On behalf of DG SANCO, 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*, Munich, May 2007 and the meeting of the groups dealing with the topics mentioned above. In particular, JAT on heat treatment chaired by Marina Nicolas.

## 6. Publications and reports

- 1: Applicability of Standard ISO 21528 methods for the enumeration of *Enterobacteriaceae* in dairy products. Alexandra CAUQUIL, Nelly AUDINET, Marie-Léone VIGNAUD, Véronique LAFARGE, Bertrand LOMBARD and Adrien ASSERE. January 2006-June 2007.
- 2: Report on Inter-laboratory Proficiency testing on Alkaline Phosphatase activity. December 2005-2006, Amended version 15 June 2007. Anne-Cécile BOITELLE et Marina NICOLAS.