



Maisons-Alfort laboratory for
food safety



EU-RL MMP

European Union Reference Laboratory for
Milk and Milk Products

2010 Technical report of the Reference Laboratory of the European Union for Milk and Milk Products

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INTRODUCTION

The Maisons-Alfort Laboratory for food safety of ANSES (French agency for food, environmental and occupational health safety) – formerly Afssa-Lerqap - has undertaken, as European Union Reference Laboratory (EU-RL) for milk & milk products (EU-RL MMP, formerly CRL MMP), 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 EU-RL for the period 2006-2011.

These actions are part of the current mandate of the EU-RL 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 EU-RL has provided in particular 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 EU-RL 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 EU-RL (Unit HMPA) has launched in 2010 an investigation study (homogeneity and stability) to find a way to stabilize sufficiently the total flora (TF) contamination of raw goat's milk. The aim of this study was to evaluate the possibility for the EU-RL to prepare and dispatch itself the samples to be used for the 2011 PT trial. Bacteriostatic agents have been tested to allow TF stabilization during transportation to the laboratory and storage. The chemical stabilizer chosen will make it possible to bacteria to grow on plates after the dilution steps.

This study will be finalised in 2011.

B. ENUMERATION OF SOMATIC CELLS

(annual)

The EU-RL (Unit HMPA) has organised in 2010 an inter-laboratory PT trial for the NRLs on the enumeration of somatic cells count (SCC) in raw cow's milk by the reference method, the Standard EN ISO 13366-1 (microscopic method). For this trial, the laboratories in charge of calibrating the instrumental methods at national level were also invited to participate.

The EU-RL (Unit HMPA) has prepared and dispatched in advance (Week 44) the instructions and the test report for the trial. ACTILAIT/CECALAIT has prepared samples (7 samples of raw cow's milk, with different levels of somatic cells) and dispatched them in December (Week 45) to the participating laboratories (28 laboratories).

The EU-RL has collected the results and is currently preparing the PT trial report which will be soon dispatched.

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 EU-RL (Unit HMPA) had launched an experimental study in 2007 using a flow cytometer (Bactocount, Bentley) as an alternative method for the enumeration of bacterial TF and SCC in raw cow's milk, in order to investigate the correlation relationship between this method and the respective reference methods, especially the influence of production factors of raw milk (mainly variation in breeds, period of lactation, type of feeding) on this conversion relationship.

In 2010, the EU-RL (Unit HMPA) has launched also this study on the analysis of the raw goat's milk. Batches of raw goat's milk were delivered at regular intervals of time and analysed for TF by the reference methods and by Bactocount. 97 samples were analyzed, received from 15 different producers of 3 departments and from three different goat's breeds.

B. DETERMINATION OF TOTAL FLORA AT 30°C IN COW'S COLOSTRUM

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

The EU-RL conducted an enquiry, launched by circular e-mail dated 09/07/2010, to collect existing national hygienic requirements/microbiological criteria on cows' colostrums. The synthesis of the enquiry showed that there is no national criterion on colostrum in most of the countries except 3 (out of 21 answers).

The outcome of this enquiry will be presented at the 2011 workshop.

1.3. COORDINATION OF THE NRL ON DETERMINATION OF TOTAL FLORA

(multi-annual)

The survey on TF determination in raw milk in European Member States dispatched in 2006 was re-circulated to the NRLs by circular letter dated 7 July 2010. The outcome was presented at the 2010 workshop. A written report will be prepared and dispatched to the NRLs.

The current practices in terms of validation of the instrumental methods according to the Standard EN ISO 16140 were discussed at the 2010 workshop.

1.4. STANDARDIZATION ON VALIDATION OF ROUTINE METHODS FOR TOTAL FLORA IN RAW MILK

(multi-annual)

The EU-RL has followed the progress in the IDF/ISO standardization works for the revision of the Standard 161/preparation of Standard ISO 16297 on the evaluation of instrumental methods for TF determination in raw milk; in particular the preparation of DIS vote, and informed the NRLs of the progress made at the 2010 workshop.

1.5. DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS FOR SOMATIC CELLS COUNT IN RAW MILK

(multi-annual)

No progress has been made in 2010 on the development of certified reference materials for SCC in raw milk, awaiting that DG SANCO contacts the JRC/IRMM (Geel) for that purpose.

1.6. DEVELOPMENT OF A REFERENCE SYSTEM FOR SOMATIC CELLS COUNT IN RAW MILK

(multi-annual)

The EU-RL participates to the IDF/ICAR Project Group in charge of developing a reference system for SCC.

In 2010, the IDF/ICAR Project Group conducted an enquiry to collect practices on the use of alternative methods for routine control of SCC in raw milk samples and on the anchoring of counting. This questionnaire was dispatched by circular letter on 17/11/2010 to the NRLs which were asked to transmit the questionnaire to the laboratories performing routine control of SCC in raw milk, in their respective Member States. NRLs have collected a large number of replies.

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 for correctly pasteurised cow's milk (350 mU/l) and conditions to use alternative methods.

In 2010, the EURL team on AP (Team CAT-AP) was audited by the French accreditation body COFRAC and was successful in maintaining its accreditation regarding AP determination in milk.

2.1. INTER-LABORATORY PROFICIENCY TESTING FOR THE NRLS

(annual)

A. PT TRIAL IN GOAT'S MILK

The EU-RL team on AP had scheduled to organize in 2009 a PT trial on the use of the reference fluorimetric method (ISO 11816-1) in goat's whole milk. This trial had to be postponed to 2010 due to the lack of sample homogeneity.

In 2010 (week 49), the EU-RL organized a PT trial dedicated to the determination of AP in goat's milk. Samples to be tested consisted of semi-skimmed, UHT goat milk at six different levels of AP, all in blind duplicates. The PT trial comprised a sufficient number of laboratories and samples to comply with the specifications of ISO 5725, parts 1 and 2, and consequently the inter-laboratory study was also evaluated as an international validation trial to generate precision figures.

The development study of a PT material with goat's milk, including the homogeneity and stability tests, had been launched in 2009 and has been completed in 2010. The report of this study has been dispatched by circular letter dated 29/03/2010. Homogeneity and stability tests were also carried during the trial.

22 NRLs participated to this PT trial. The report of this PT trial is in the process of drafting and will be soon circulated to NRLs.

B. PT TRIAL ON COW'S MILK CHEESES

The EU-RL team on AP organized a PT trial (Week 18-19), and a remedial session, on AP determination in different types of cow's milk cheese. Soft, semi-hard and hard cheese samples with a wide range of AP activities were submitted to the trial.

The aim of this PT trial was to assess:

- the ability of NRLs to apply correctly, and in a harmonized way, the protocol prescribed by the EU-RL;
- the ability of NRLs to correctly distinguish cheese made from pasteurised milk from cheese made from non-pasteurised milk.

19 NRLs participated to this PT trial, whose report is to be soon dispatched.

2.2. ANALYTICAL DEVELOPMENT

(multi-annual)

A. FLUOROPHOS REAGENTS AND INSTRUMENT

In 2009, the EU-RL had compiled comments and remarks received by NRLs on problems experienced using the instrument and/or the reagents prescribed in the EU method for the official control of alkaline phosphatase in pasteurized milk (ie. ISO 11816-1). Comments were addressed and discussed with Advanced Instruments, which provides the instrument and reagents and it had been decided that the better way to communicate on this topic was the organization of an "educational session" at the EU-RL.

The educational session was organized on 18 March 2010. A half-day session was dedicated to an interactive talk by Advanced Instruments on "good use of the instrument/material/controls/reagents, critical points, maintenance etc" and another half-day (afternoon) session consisted in a practical demonstration of the theoretical aspects developed in the morning.

B. DETERMINATION OF THE PHOSPHATASE ACTIVITY IN OTHER THAN COW'S MILK

CAMEL'S MILK

The Central Veterinary Research Laboratory (CVRL) in Dubaï launched a study on the identification of adequate pasteurisation markers in camel's milk, in collaboration with the German NRL.

In 2010, the EURL made a detailed critical review on preliminary results forwarded by CVRL in a report addressed to DG Sanco by e-mail of 28 January.

There has been neither follow-up nor feed-back on this issue later in the year.

GOAT'S AND EWE'S MILK

Given the delay in the organization of the PT trial on goat's and ewe's milk, the studies on goat's and ewe's milk are postponed. In addition, EU-RL means have been reallocated to other works, compared to the 2010 work programme, in particular:

- Development study of a PT material of goat's milk for PT trial, much heavier than scheduled, see 2.1.A;
- Organization of a 2nd PT trial, see 2.1.B;
- Organization of a training session on AP determination in cheese, see C).

C. DETERMINATION OF ALKALINE PHOSPHATASE IN CHEESES

Following an ISO/IDF international meeting on heat treatment in autumn 2009, some further suggestions were introduced by the experts aiming to improve the analytical protocol. The amendments proposed were investigated in the EU-RL laboratory in 2010 and were integrated, when pertinent, by the EU-RL as ISO/IDF Project Leader in the draft project for determination of AP in cheese.

This draft method for cheese was submitted by the EU-RL to ISO/IDF on 22 April 2010. This draft was also dispatched to the NRLs on 23 April 2010.

A training session on AP determination in cheese with the most recently up-dated version of the protocol was organized by the EU-RL on 19 March 2010. 11 NRLs participated to this training session.

D. COMPARISON OF THE CHEMILUMINESCENT/FLUORIMETRIC METHODS

The developer of the chemiluminescent method (Charm) needed time in 2010 to work on correction of the calibrator so as to correct the positive bias that appeared over time relative to Fluorophos.

The need that EU-RL continues equivalence evaluations with this new calibrator in 2011 was confirmed.

E. REACTIVATED PHOSPHATASE

The EU-RL became member of the new IDF/ISO project group on reactivated phosphatase and contributed to the bibliographic review.

3. ASSISTANCE TO THE NRL

No individual training, other than training sessions for AP determination (see 2.2.A & C).

4. NRLS WORKSHOP

The EU-RL organised on 30 September & 1 October 2010 the 13th NRL workshop dedicated to total flora in raw milk. In particular, this workshop has enabled:

1. to make a point of works undertaken by the EU-RL since the workshop dedicated to TF in 2006;
2. to envisage the work programme for the following years in this area.

The report was dispatched to the NRLs by circular letter dated 30 November 2010.

5. TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION

(multi-annual)

5.1. DG SANCO ACTIVITIES

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

- Participation to the bilateral Canada/UE negotiations on equivalence of sanitary measures: oral presentation of B. Lombard at the meeting of 18 February 2010 on relative risks related to raw milk soft cheeses.
- Participation to the bilateral US/UE negotiations on veterinary agreement on milk hygiene:
 - o Drafting of a background note on AP determination in cheeses (note dated 04/07/2010);
 - o Oral presentations at the meeting of 8 July 2010 on:
 - Choice/equivalence of methods for official control of AP in cheeses and export of cheeses to the USA (M. Nicolas),
 - Sanitary relevance of SCC and TF criteria in the European legislation (B. Lombard).
- Drafting of a note on *Pseudomonas* in dairy products, in particular cheeses, public health relevance (note dated 28 June 2010).
- Drafting of a note on the Australian submission for equivalence of alternative measures to foremilk stripping (note dated 20 December 2010).

Further to the exchanges at former workshops and the request to DG SANCO of the EU-RL, on behalf of the network of NRLs, the Regulation 365/2010 amending the Regulation EC 2073/2005 on microbiological criteria for food, modifying the criterion on *Enterobacteriaceae* in pasteurised milk and other pasteurised liquid dairy products, has been published. This amendment enables for this criterion the use as reference method of the

colony-count technique (ISO 21528-2) instead of the most probable number technique (ISO 21528-1).

5.2. 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:
 - somatic cells count: reference and alternative methods, reference system,
 - total flora: alternative methods,
 - phosphatase test: reference and alternative methods, and other pasteurisation tracers.
2. The 2010 IDF/ISO Analytical Week (Montréal, 17-22 May 2010) and in particular the meetings of the groups dealing with the heat-treatment.

6. REPORTS DISPATCHED IN 2010

1. EU-RL for MMP/AFSSA/LERQAP/CAT AP/2010/01, Report on the homogeneity tests on goat's whole milk prior to the organisation of a proficiency testing trial on alkaline phosphatase determination. 20/03/2010. Caroline VIGNAUD, Anne-Cécile BOITELLE, Marina NICOLAS from Unit Characterisation of toxins, Team Alkaline Phosphatase (CAT-AP)
2. *EU-RL method for the determination of the alkaline phosphatase activity in cheese.* 22/04/2010.
3. Report of the 2009 inter-laboratory proficiency testing (PT) trial dedicated on the enumeration of total flora in raw cow's milk. 27/07/2010.
4. Report of the comparative study of different bacteriostatic agents for raw cow's milk samples used in interlaboratory trials for the enumeration of microorganisms at 30°C. 13/07/2010. Alexandra CAUQUIL.
5. Report of the 2010 workshop dedicated to Total Flora. 30/11/2010