anses
French agency for food, environmental and occupational health safety

Maisons-Alfort laboratory for food safety

EURL MMP
European Union Reference Laboratory for Milk and Milk Products

2013 Work Programme of the European Union Reference Laboratory 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) foresees to undertake, as European Union Reference Laboratory for Milk & Milk Products (EURL MMP), the following works in 2013, according in particular to the actions planned at the 14th Workshop of the National Reference Laboratories (NRLs) (2&3 May 2011).

These actions are part of the current mandate of the EURL 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 EC Regulation 853/2004 *laying down specific hygiene rules for food of animal origin*.

The Annex III, Section IX of EC 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:
    - 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);
    - 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:
    - 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.
    - 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 EURL foresees in particular to provide a support to the NRLs for the implementation of:

- the EC Regulation 853/2004;
- the derived EC Regulation 2074/2005 (modified by EC Regulation 1664/2006) defining amongst other the testing methods for raw milk and heat-treated milk to be used by competent authorities and food business operators:
    - 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,
    - 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 1: In brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2013), either multi-annual (on-going programme on several years).

NB 2: The activities are gathered according to the tasks allocated to EURLs, defined by EC Regulation 882/2004 on official controls (Article 32, paragraph 1 on EURLs for feed and food):

- *Section 1: Dispatch of methods and proficiency testing trials for the NRLs,*
- *Section 2: Analytical development,*
- *Section 3: Training of the NRLs,*
- *Section 4: Technical and scientific assistance to the European Commission.*

0 GENERAL ASPECTS

0.1 GENERAL COORDINATION (MULTI-ANNUAL)

General coordination by the EURL (management team, administrative PAFT department), of the network of the NRLs (dispatch of circular letters and documents, coordination of the scientific and technical support to NRLs, …).

Relations with DG SANCO, coordination of the scientific and technical advice to DG SANCO, management of annual contract with DG SANCO (annual budgets and work programmes, annual technical and financial reports).

In-house follow-up of EURL activities, expenses, support to laboratory units involved in EURL activities.

0.2 WORKSHOP OF THE NRLS (ANNUAL)

The EURL will organise in 2013 the 16th NRL Workshop. This workshop will aim in particular:

- to provide an update of the work undertaken by the EURL since the last workshop;
- to establish a framework programme for the future.

Three experts would be invited, as well as NRLs from accessing countries.

0.3 SCIENTIFIC MONITORING AND COMMUNICATION (MUTI-ANNUAL)

The EURL teams will conduct scientific monitoring in its area of competence, as well as communicate on the works conducted as EURL MMP, disseminate the outcome of works in the international scientific community, in particular through drafting of written publications, oral presentations and posters to international symposia.

1 DISPATCH OF METHODS AND PROFICIENCY TESTING TRIALS

1.1 HYGIENE OF RAW MILK

1.1.1 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY PROFICIENCY TESTING TRIALS ON TOTAL FLORA IN RAW COW'S MILK (MULTI-ANNUAL)

Duration: start: 2012- expected end 2014

Objective
In 2009, the EURL MMP had conducted an investigation study (homogeneity and stability) which had enabled to select a chemical agent (boric acid), which stabilized sufficiently total flora (TF) levels of raw cow's milk to organize proficiency testing (PT) trials for the NRLs. The purpose of the on-going study is to optimize the protocol currently used, initially defined in 2009.

Expected output and time of delivery
The EURL MMP (Unit EDB) will continue in 2013 an investigation study launched in 2012 on the homogeneity and stability of total flora (TF) in raw cow's milk samples.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

1.1.2 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY PT TRIALS ON TOTAL FLORA IN RAW SHEEP'S MILK (MULTI-ANNUAL)

Duration: start: 2013- expectedend: 2015

Objective
This study aims at finding a way, such as the addition of a chemical agent, to stabilize sufficiently TF level of raw sheep's milk, in order to organize PT trials for the NRLs on TF in raw sheep's milk. It is intended to select a formula adapted to TF; formula which would allow the bacteria to grow on plates after the dilution steps.

Expected output and time of delivery
The EURL MMP (Unit EDB) will launch in 2013 this investigation study, assessing in particular stability and homogeneity.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

1.1.3 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY PT TRIALS ON SOMATIC CELLS IN RAW COW'S MILK (MULTI-ANNUAL)

Duration: start: 2011- expected end: 2013

Objective

The aim of this on-going study is to find how to stabilize sufficiently the somatic cell contamination of raw cow's milk by using a new chemical agent, in order to organize PT trials for the NRLs on somatic cell count (SCC) in raw cow's milk.

Expected output and time of delivery

The EURL MMP (Unit EDB) will complete in 2013 the investigation study on homogeneity and stability of SCC in raw cow's milk samples.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

1.1.4 SOMATIC CELL COUNT IN RAW COW'S MILK (ANNUAL)

The EURL MMP (Unit EDB) will organize in 2013 an inter-laboratory PT trial on SCC in raw cow's milk by the reference method, the Standard EN ISO 13366-1 (microscopic method).

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

1.1.5 COORDINATION ACTIVITIES ON ANALYSIS OF TOTAL FLORA AND SOMATIC CELLS (MULTI-ANNUAL)

Duration: start: 2006- expected end: not yet defined

Objective

EC Regulation 2074/2005 (modified by EC Regulation 1664/2006) defines the testing methods for raw milk to be used to check compliance with the limits for total flora and somatic cells count laid down in Regulation 853/2004: either the reference methods (EN ISO 4833 for TF or EN ISO 13366-1 for SCC), or alternative methods, under certain conditions detailed in EC Regulation 2074/2005.

Given the workload to implement the reference methods for TF and SCC, routine controls (own checks) are currently performed in Europe in overwhelming majority -if not uniquely- by alternative methods, mostly by instrumental methods, based on flow cytometry. The results of the instrumental methods have to be converted into the unit of the reference methods, to check compliance with legal limits of EC Regulation 853/2004, expressed in the units of the reference methods. This requires the establishment of a conversion equation between the instrumental and the reference methods, which has been identified as a critical point for the implementation of the instrumental methods.

The EURL MMP (Unit EDB) is conducting the harmonization of conversion equations between the alternative methods and EN ISO 4833 reference method, for TF in raw milk.

Expected output and time of delivery

After a first step of harmonization of conversion equations at national level, under the responsibility of NRLs and coordination by the EURL (launched in 2006, almost completed), the current second step is to investigate the possibility of conversion equation harmonization at European level. The EURL will conduct this work in collaboration with a working group of volunteering NRLs, settled in 2012 and which first met on 3 October 2012.

In addition, the EURL will follow the conduction and outcome of validation studies by certification bodies (Afnor Certification, MicroVal) of instrumental methods for TF in raw milk. The EURL will also follow the launching of validation studies by these certification bodies on instrumental methods for SCC in raw milk, once the EURL document on validation criteria will be developed (see 1.1.6).

Finally, the EURL will follow the final steps of the revision of IDF 161A on the validation of instrumental methods for TF in raw milk.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): a & c

1.1.6 CRITERIA FOR THE VALIDATION OF ROUTINE METHODS FOR SOMATIC CELL COUNT IN RAW MILK (MULTI-ANNUAL)

Duration: start: 2012- expected end: 2013

Objective

According to EC Regulation 2074/2005 (modified by EC Regulation 1664/2006), the use of alternative methods to the reference method EN ISO 13366-1 for SCC in raw milk is possible if they are validated against EN ISO 13366-1 in accordance with the protocol of the Standard EN ISO 8196 or other similar internationally accepted protocols.

Up to now, no real complete validation study according to EN ISO 8196 has been performed on alternative methods, mainly instrumental methods, for SCC in raw milk.

Expected output and time of delivery

In 2013, the EURL MMP (Unit EDB) will finalize a document defining criteria for the validation of instrumental methods, for SCC in raw milk (first draft prepared in 2012 and presented at the annual workshop). This document would have a similar structure than the one already written for TF in raw milk.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): a & c

1.2 PASTEURIZATION TRACERS OF MILK AND MILK PRODUCTS

1.2.1 INTER-LABORATORY PT TRIAL ON THE DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY IN CHEESE (ANNUAL)

Objective

The EURL MMP (Team CAT-AP) conducts works on the determination of alkaline phosphatase (AP) activity in cheese, in the perspective of expanding pasteurization criteria to dairy products other than milk, so as to support DG SANCO in setting a legal limit at European level for AP activity in cheeses made from correctly pasteurised cow's milk (see 2.2.1 and 4.1.1).

The EURL will organize in 2013 an inter-laboratory PT trial on determination of AP activity in cheese by the fluorimetric method (EURL in-house method and draft CEN/IDF/ISO Standard method).

Expected output and time of delivery

a) As a preliminary step of the 2013 PT trial, the EURL will organize a study, with a limited number of NRLs, to assess the impact of the "nature" of sample type that will be included in the PT trial. Cheese samples as commercially available and the same samples prepared by EURL according to the clause on preparation of the test sample of the EURL in-house method, will be sent to partner NRLs. The EURL will scrutinize results obtained so as to evaluate the contribution of the "nature" of the sample types to the overall variability of the results. The EURL will also study the homogeneity and stability of AP in both the commercial samples as such and on the latter after the test samples have been prepared by EURL.

   Based on the outcome of this study, the EURL will decide whether commercial samples or samples already prepared by EURL will be dispatched to NRLs for the PT trial. See also the study described in 4.1.1 which will help for the choice of the PT samples. The EURL will conduct homogeneity and stability tests, aligned with the PT trial.

   The EURL will include soft cheese (surface mould/camembert type and washed-rind cheeses), semi-hard cheese and hard cheese in the preliminary study and the PT trial.

b) Once the preliminary study finalized, the EURL will organize for the NRLs the inter-laboratory PT trial on the fluorimetric determination of AP activity in cheese. The protocol to apply will be the EURL in-house method, also progressed through CEN and IDF/ISO standardization procedures to become an EN IDF/ISO Standard (see 2.2.1), that the EURL will dispatch to NRLs.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

2 ANALYTICAL DEVELOPMENT

2.1 HYGIENE OF RAW MILK

2.1.1 DETERMINATION OF TOTAL FLORA AT 30°C AND SOMATIC CELLS IN RAW MILK BY AN INSTRUMENTAL METHOD (MULTI-ANNUAL)

Duration: start: 2007- expected end: 2015

Objective

See 1.1.5 for the context of this project.

The EURL MMP (Unit EDB) is conducting an experimental study on raw cow's and goat's milks, using a flow cytometer (Bactocount) purchased in 2007, as an alternative method to the reference methods (bacterial count for TF and microscopic method for 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 equation (variation in breeds, period of lactation, type of feeding ...). This study could in particular help NRLs to correctly identify the different factors which can have an impact on the conversion factor. This study is also needed to harmonize conversion equation at national or European level (see 1.1.5).

Expected output and time of delivery

In 2013, for raw cow's and goat's milk, the EURL will continue the study of the influence of certain factors (such as environment of animals, storage of milk) on the conversion equation. With a suitable experimental design, these factors will be controlled.

To conduct this study, batches of raw cow's and goat's milk will be delivered at regular intervals of time (transport of samples to EURL sub-contracted) and will be analysed by the EURL in parallel by the reference methods and by the Bactocount method for TF and SCC.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): c

2.1.2 DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS FOR SOMATIC CELL COUNT IN RAW MILK (MULTI-ANNUAL)

Duration: start: 2012- expected end: 2016

Objective

Given the deficiencies of the reference microscopic method for SCC in raw milk (lack of reproducibility of EN ISO 13366-1 Standard method) and the limited number of laboratories using it, it is of utmost importance to develop Certified Reference Materials (CRMs) to

ensure the reliability of SCC analyses of raw milk in Europe, either using the microscopic reference method or instrumental alternative methods. Such CRMS are not currently available. The need to develop CRMs for SCC was highlighted several times by the network of NRLs MMP.

CRMs are needed to calibrate instrumental methods, mostly used for routine analyses of SCC in raw milk, as to have comparable SCC analyses conducted within each European country and between different European countries.

Expected output and time of delivery

On 11 May 2012, a meeting has been organised with DG SANCO, EC/JRC/IRMM (Geel, BE) and EURL MMP to investigate the need and the feasibility to produce CRMs on SCC in milk. Another meeting has been held at IRMM with IDF/ISO and EURL MMP to further investigate the needs and feasibility of these CRMs.

In 2013, JRC, in collaboration with EURL MMP, will progress to assess the economic and technical feasibility to produce CRMs:

- A questionnaire will be drafted by EURL MMP and IDF/ISO to quantitatively estimate the needs of CRMs. EURL MMP will circulate this questionnaire to the NRLs to estimate their needs, as well as of the network of milk control laboratories in each European country.
- In case of a positive outcome of this enquiry (level of need considered sufficient by IRMM), EURL MMP (Unit EDB) would launch a feasibility study to prepare CRMs with lyophilized milk.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): b

2.2 PASTEURIZATION TRACERS OF MILK AND MILK PRODUCTS

2.2.1 ALKALINE PHOSPHATASE ACTIVITY IN CHEESE (MULTI-ANNUAL)

Duration: start: 2008- expected end: 2014

Objective

See 1.2.1 which introduces the EURL works on alkaline phosphatase (AP) activity in cheese, together with the frame and objectives of these works.

More specifically, this project aims at developing and validating the reference method that would be associated to the legal limit on AP in cheeses made from pasteurized milk. In addition, this topic has been on the agenda of the meeting on EU/US dairy issues (Brussels, 8 July 2010), more precisely the issue of the method for AP determination to be used for EU exports of pasteurized milk cheeses to US (lack of consensus between Europe and US on the choice of the method to be used).

Expected output and time of delivery

The EURL MMP (Team CAT-AP) is progressing in the development of a method for the determination of AP activity in cheese, in parallel through the CEN and IDF/ISO standardization procedures. The expected end-product is a EN/ISO-IDF International Standard, for which the EURL acts as Project Leader.

In 2013, EURL MMP, as IDF/ISO Project Leader, will organize an international validation inter-laboratory study on the draft Standard method, which will be progressed to a Draft International Standard (DIS).

A large participation from NRLs to the inter-laboratory validation study is expected but participation from international laboratories beyond UE will also be seeked so that the standard achieves a wide international recognition.

It is important to note that the validation inter-laboratory trial, although it will be treated independently and will be evaluated as a totally distinct project, will be run by EURL in parallel with the PT trial mentioned under 1.2.1 and will thus result in no additional cost.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): c

2.2.2 HEAT TRACERS OTHER THAN ALKALINE PHOSPHATASE: DEVELOPMENT OF ANALYTICAL TOOLS (MULTI-ANNUAL)

Duration: start: 2007- expected end: 2016 (GGT assays)

Objective

Correct pasteurization of milk & milk products from other species than cow needs to be checked, according to EC Regulation 853/2004.

DG SANCO has specifically requested the EURL MMP to work on markers of pasteurization treatment for camel milk (see letter dated 21/12/2007).

The EURL MMP (Team CAT-AP) is a partner to an international ISO/IDF project (Project Leader from US/FDA) aiming to identify alternative pasteurisation tracers when AP has shown not to be a pertinent indicator and to develop analytical tools for their determination.

Gamma-glutamyl transpeptidase (GGT) is another enzyme than AP found in milk, with a slightly higher denaturation temperature than AP. Developing a method for GGT would provide an alternative thermal enzyme marker for dairy products where AP has not shown to be a pertinent indicator. For example GGT has been demonstrated to be a useful thermal enzyme marker for heat treatment of camel milk.

Expected output and time of delivery

To evaluate and, if needed, amend the protocol introduced by the FDA expert, the EURL will conduct preliminary assays in 2013. EURL will apply the method as described in the FDA

document and as submitted to the IDF/ISO experts in order to verify how it works and if it needs to be clarified or optimised.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): c

3 TRAINING OF THE NRLS

3.1 HYGIENE OF RAW MILK (ANNUAL)

The EURL MMP (Unit EDB) will organize in 2013 a second training session for the NRLs on the SCC in raw cow's milk, with the reference method EN ISO 13366-1.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): d

3.2 PASTEURIZATION TRACERS OF MILK AND MILK PRODUCTS (ANNUAL)

No group training is scheduled but the EURL MMP (Team CAT-AP) will follow-up with any individual request from the NRLs.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): d

4 TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION (MULTI-ANNUAL)

4.1 PASTEURIZATION TRACERS OF MILK AND MILK PRODUCTS

4.1.1 SURVEY ON ALKALINE PHOSPHATASE ACTIVITY IN CHEESES MADE FROM PASTEURIZED COW'S MILK, AS TO SET A EUROPEAN LEGAL LIMIT (MULTI-ANNUAL)

Duration: start: 2008- expected end: 2014

Objective

See 1.2.1 which introduces the EURL works on alkaline phosphatase (AP) activity in cheese, together with the frame and objectives of these works.

In this context, the EURL MMP has been conducting over the last 3 years a European study aiming at generating data on the content of residual AP in pasteurised cow milk cheese in the different EU Member States.

Expected output and time of delivery

In 2013, the EURL MMP (Team CAT-AP) will continue the coordination of the work performed by NRLs on soft, hard and semi-hard cheeses made from cow's pasteurised milk.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): e

4.1.2 FIXATION OF A EUROPEAN LEGAL LIMIT ON AP IN EWE'S PASTEURIZED MILK (MULTI-ANNUAL)

Duration: start: 2013- expected end: 2016

Objective
The purpose of this multi-annual project is to support DG SANCO in its request to prescribe a legal limit of AP activity in ewe's pasteurised milk.

Expected output and time of delivery

In 2013, the EURL MMP (Team CAT-AP) will work on the establishment of AP inactivation curves in ewe's milk and specifically on 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.

Subsequently (in 2014 and beyond), the EURL would initiate work on raw and laboratory-pasteurised milk and would coordinate declination of the project at the NRLs' level.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): e

4.2 DG SANCO ACTIVITIES (MULTI-ANNUAL)

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

- Participation to the bilateral US/UE negotiations on veterinary agreement (dairy hygiene, total flora, somatic cell count and phosphatase activity);
- And any other question which may arise during the year;
- Including the participation of a EURL MMP representative to 2 meetings
- Missions: 2 meetings at EC, Brussels.

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): e

4.3 PARTICIPATION TO ISO/IDF STANDARDIZATION WORKS (MULTI-ANNUAL)

On behalf of DG SANCO (and nomination as EC representative), participation to:

1. The IDF/ISO works on the analytical methods specific to the analysis of milk and milk products in the mandate of the EURL MMP:
   - somatic cells count: reference and alternative methods,
   - total flora: alternative methods,
   - determination of alkaline phosphatase and other pasteurisation tracers in milk and milk products: reference and alternative methods,
   - statistical & sampling aspects;
2. The 2013 IDF/ISO Analytical Week (Rotterdam, The Netherlands, June 2013) and the meetings of the groups dealing with the topics mentioned above
   - Mission of 3 EURL representatives:
     - Veronique DFEPERROIS for groups on somatic cells count and total flora,
     - Marina NICOLAS for groups on AP and other pasteurization tracers,
     - Bertrand LOMBARD for statistical and sampling aspects, liaison with ISO/TC 34/SC 9 (standardization in food microbiology, including milk and milk products).

Relation to EURL specific tasks (article 32.1 of EC Regulation 882/2004): e