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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

2014 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 2014, according in particular to the actions planned at the 15th Workshop of the National Reference Laboratories (NRLs) (3-5 October 2012).

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:

(i) the EC Regulation 853/2004 modified *laying down specific hygiene rules for food of animal origin*

(ii) the EC Regulation No 854/2004 *modified laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption*, dealing in Article 8 and Annex IV with raw milk and dairy products. Annex IV requires that the (national) competent authority monitors the checks carried out in accordance with Annex III, Section IX, Chapter I, Part III to Regulation (EC) No 853/2004.

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 2074/2005 (modified by EC Regulation 1664/2006) defining in Article 6a and Annex VIa 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 2014), 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 SAG 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 2014 the 17th 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.

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) intends to complete in 2014 an investigation study launched in 2012 of raw cow's milk samples, to be used as material for PT trials on TF, assessing in particular their homogeneity and stability.

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- expected end: 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 continue in 2014 the investigation study of raw sheep's milk samples, to be used as material for PT trials on TF, assessing in particular their 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: 2014

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 2014 the investigation study of raw cow's milk samples, to be used as material for PT trials on SCC, assessing in particular their homogeneity and stability.

Mission
EURL will visit the Belgian NRL to collaborate on the mode of stabilization of samples for PT trials for SCC (1 day, Melle, BE).

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

1.1.4 ENUMERATION OF TOTAL FLORA AT 30°C IN RAW SHEEP'S MILK (ANNUAL)

The EURL MMP (Unit EDB) will organize in 2014 an inter-laboratory PT trial on enumeration of total flora at 30°C in raw sheep's milk, with the Standard method EN ISO 4833.

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

1.1.5 COORDINATION ACTIVITIES ON ANALYSIS OF TOTAL FLORA (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 continue this work in 2014 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 MicroVal of 2 instrumental methods for TF in raw milk.

Finally, the EURL will follow the revision by IDF/ISO of EN ISO 21187/ IDF 196, which provides guidance for establishing and verifying a conversion relationship between routine method and reference method.

Meeting
EURL will organize a meeting of the NRL WG (max 8 participants, 2 days).

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

1.1.6 COORDINATION ACTIVITIES ON ANALYSIS OF SOMATIC CELLS

Duration: start: 2013- expected end: not yet defined

Objective, expected output and time of delivery

The EURL will follow the launching of validation studies by a certification body on instrumental methods for SCC in raw cow's milk.

The validation studies will be conducted in accordance with the EURL MMP document defining criteria for the validation of instrumental (epifluorescent) methods for the enumeration of somatic cells in raw cow's milk. This document was finalized at the beginning of 2013 and was sent to the certification bodies (Afnor Certification, Microval), as well as to providers of instrumental methods for SCC.

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)

Duration: 2014

Objective

The EURL will assess, through this PT trial, the individual performance of NRLs to determine alkaline phosphatase (AP) in cheese.

Expected output and time of delivery

In 2014, EURL will organize an 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 (EN ISO 11816-2, see 2.2.1), that the EURL will dispatch to NRLs.

Different types of cheese, at different levels of AP will be distributed for the PT and EURL will conduct homogeneity and stability tests.

A report will be drafted and circulated to participating NRLs in due time.

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, 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 2014, 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) and will be analysed by the EURL in parallel by the reference methods and by the Bactocount method for TF.

Sub-contracting
Transport of raw milk samples to EURL will be sub-contracted.

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.

EC/JRC/IRMM (Geel, BE), in collaboration with EURL MMP, envisages to develop such CRMs.

Expected output and time of delivery

In 2013, JRC/IRMM, in collaboration with EURL MMP and IDF/ISO/ICAR, has been assessing the economic and technical feasibility to produce CRMs on SCC in milk. For that purpose, a questionnaire was drafted by EURL MMP and IDF/ISO/ICAR to quantitatively estimate the needs of CRMs. EURL MMP has circulated this questionnaire to the NRLs to estimate their needs, as well as of the network of milk control laboratories in each European country. The outcome of this enquiry was presented at the IDF/ISO Analytical Week in June 2013 and showed the clear interest to have CRMs for SCC in milk.

In 2014, at the condition that JRC/IRMM decides to launch the project of developing CRMs on SCC in milk, EURL MMP (Unit EDB) intends to launch, in collaboration with JRC and IDF/ISO/ICAR, a feasibility study to prepare CRMs with lyophilized milk.

Mission
1 meeting at JRC/IRMM, Geel, 2 days.

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

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 4.1.1).

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 standardization 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 an EN/ISO-IDF International Standard (EN ISO 11816-2), for which the EURL acts as Project Leader.

In 2014, EURL MMP, as IDF/ISO Project Leader, will organize the second part of the international validation inter-laboratory study on the ISO Committee Draft Standard method, which will be progressed to a Draft International Standard (DIS). (First part of the validation study: Autumn 2013). A large participation from NRLs to the inter-laboratory validation study is expected but participation from laboratories beyond UE will also be seeked so that the future Standard method 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.

Missions
Visit of the German NRL on the AP determination in cheese (Kiel, Germany, 1 day).

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

Objective

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

The initial proposal of the US project leader was to consider GGT as an alternative pasteurization tracer to AP for milk and cheese in general (milk and milk products from cow, goat, sheep, camel etc). The EURL disagreed with this proposal because GGT, having a higher denaturation temperature than AP, there is a real risk of obtaining false positive results if cheeses are tested for GGT instead of AP, thus introducing a possible barrier to trade for European cheeses made for pasteurized milk. EURL was nevertheless in favor of developing a method for GGT as a heat marker for dairy products *where AP has not shown to be a pertinent indicator* (ie. for camel – mare – llama etc. milk and milk products).

The arguments developed by EURL were accepted and the initial draft was amended accordingly: a GGT method is under development so as to provide an appropriate alternative thermal enzyme tracer for dairy products where AP is irrelevant.

Expected output and time of delivery

In 2014, EURL will participate to a scheduled pre-collaborative study on GGT activity determined by the microwell fluorimetric method (FDA document).

Further to that, EURL will undertake a study to compare GGT activities versus AP, the latter being the official criterion for pasteurized milk EU controls (fluorimetric method) on samples prepared at laboratory scale ranging from thermization to high pasteurization.

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

2.2.3 REACTIVATED PHOSPHATASE AND DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL

No experimental work is on the EURL work programme but the EURL will continue to exchange views with scientific experts of the US/FDA competent laboratory and other EU experts (NL-NRL, CH-NRL, ...).

3 TRAINING OF THE NRLS

3.1 HYGIENE OF RAW MILK (ANNUAL)

The EURL MMP (Unit EDB) will organize in 2014 a 3rd training session for the NRLs on 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 in 2014 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 2.2.1 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 steering 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 2014, the EURL MMP (Team CAT-AP) will complete the coordination of the work performed by NRLs on cheeses made from cow's pasteurised milk and will draft a preliminary report.

Because cheese products are submitted to international trade and undergo, among others, analytical controls related to export and import regulations, and considering that EU Member States export outside of Europe, a similar project, led by EURL, and broadened to the international level is also underway. Cow pasteurized cheeses will be shipped to EURL by Japan, New Zealand, US and possibly Brazil, for AP determination, so as to enrich the "AP data bank".

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, scientific and technical assistance on any question which may arise during the year.

Missions

2 meetings at EC, Brussels (1 day each).

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, conversion relationship
   - determination of alkaline phosphatase and other pasteurisation tracers in milk and milk products: reference and alternative methods,
   - statistical & sampling aspects;
2. The 2014 IDF/ISO Analytical Week (Berlin, Germany, May 2014) 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 (conversion relationship),
     - 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