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Maisons-Alfort laboratory for food safety

EURL MMP

European Union Reference Laboratory for Milk and Milk Products

2015 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 safetyforesees to undertake, as European Union Reference Laboratory for Milk & Milk Products (EURL MMP), the following works in 2015, according in particular to the actions planned at the 15th Workshop of the National Reference Laboratories (NRLs) of general scope (3-5 October 2012) and the 16th Workshop dedicated to pasteurization tracers (3-4 October 2013).

The scientific and technical activities of EURL MMP are mainly undertaken in the laboratory by the Milk Team of the Unit Staphylococci, *Bacillus*, Clostridia and Milk (SBCL).

The following work program is scheduled on the basis of a normal yearly activity, assuming that the renovation of the laboratory premises, planned at the beginning of 2015, will enable to implement all the experimental activities planned in 2015.

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 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 2015 the 18th NRL Workshop at Maisons-Alfort, FR. This workshop will be of general scope, on the whole work area of EURL.

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 COUNTING OF SOMATIC CELLS IN RAW COW'S MILK (ANNUAL)

EURL MMP will organize in 2015 an inter-laboratory PT trial on counting of somatic cells in raw cow's milk, with the Standard reference method EN ISO 13366-1. EURL will perform the homogeneity and stability analyses of the samples prepared for this PT trial, using an instrumental method for SCC determination.

Equipment

EURL MMP needs to purchase a new flow cytometer, dedicated only to SCC analyses (see 2.1.2).

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

1.1.2 HARMONIZATION AT EUROPEAN LEVEL OF CONVERSION EQUATIONS FOR TOTAL FLORA (MULTI-ANNUAL)

Duration: start: 2012- expected end: 2016

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 4833-1 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.

EURL MMP is conducting the harmonization of conversion equations between the alternative methods and EN ISO 4833-1 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, the current second step is to investigate the possibility of conversion equation harmonization at European level. This work is conducted by EURL in collaboration with volunteering NRLs, gathered in a working group convened by EURL.

In 2013/2014, 5 NRLs have provided data from their country. At the WG meeting of 1 October 2014 and the subsequent workshop (1-3 October), it has been agreed that collection of data would be extended to July 2015 to collect data from other MSs as to have a more representative picture at European level.

The expected output for 2015 is to complete the collection of data from a larger number of MSs, to perform a statistical analysis of the data collected, to assess the feasibility of deriving a common conversion equation at European level, and to discuss the outcome with the NRL WG then with all NRLs.

The final output of this work would be to establish a common conversion equation per instrumental method and per animal species (cow, ewe and goat), at the condition that the outcome of the data collection and its statistical analysis would show a common conversion equation is feasible.

Meeting

Half day meeting of the NRL WG in 2015, combined with the annual workshop (max 8 participants).

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 COW MILK (ANNUAL)

Duration: 2015

Objective

EURL MMP will assess, through this PT trial, the performance of NRLs to determine alkaline phosphatase (AP) activity in cow milk.

Expected output and time of delivery

In 2015, EURL will organize an inter-laboratory PT trial on the fluorimetric determination of AP activity in cow's milk. The protocol to apply will be the method, prescribed for official controls, i.e. the International Standard EN ISO 11816-1: 2013.

Cow's milk at different AP levels 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 IN RAW MILK BY AN INSTRUMENTAL METHOD (MULTI-ANNUAL)

Duration: 2007- 2015

Objective

EURL MMP has been 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 1.1.2).

Expected output and time of delivery

In 2014, the study on raw goat's milk would be completed.

In 2015, depending on the results obtained in 2014 on the possibility to establish a relationship between environmental factors and TF count, EURL MMP would carry on further experiments for raw cow's milk.

To further conduct this study, if appropriate (see before), batches of raw cow's milk would be delivered at regular intervals of time and would be analyzed by EURL in parallel by the reference method 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: 2012- 2017

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.

In a letter dated 16 July 2014, IRMM indicated its decision to launch the development of CRMs for somatic cell counting in milk, as announced during the IDF/ISO Analytical Week in June 2014. IRMM will include this project into its 2015 work programme.

Expected output and time of delivery

In 2015, this project will start with a feasibility study on (i) small scale test batches and (ii) large scale pilot batches. The aim of this feasibility study is to define how to process a suitable material, to check homogeneity, stability, commutability. A successful feasibility study is a prerequisite for producing a RM batch. The timeline for all the work of the feasibility study is January – December 2015.

The analyses listed below are referring to the feasibility study, which involves a study of small scale test batches (task 1) and a study of larger scale pilot batches (task 2):

- Task 1 (January – March 2015): study of small scale test batches (few litres each). This study will investigate in particular existing versus optimised freeze-drying programme, alternative preservation techniques.
  During this task, IRMM will ask EURL MMP to analyse 180 samples with the reference method EN ISO 13366-1.
- Task 2 (April – November 2015): study of larger scale pilot batches, at 2 levels (low and high).
  During this task, IRMM will ask EURL MMP to perform analyses for:
  - homogeneity study: 200 analyses with instrumental method per level;
  - short-term stability study: 80 analyses with instrumental method per level;
  - long-term stability study: 80 analyses with instrumental method per level;

- commutability study of the pilot batches with both applied methods:
  80 analyses with the reference method and 80 analyses with the alternative method, per level.

In total, this will amount to 340 analyses using the reference method and 880 analyses using the alternative method, to be undertaken by EURL.

Financing

For the feasibility study to be conducted in 2015:

- development and preparation of candidate RM (sampling, freeze drying, bottling and dispatching) will be supported by IRMM,
- staff and consumable costs for the analyses to be performed by EURL (see before) will be supported on EURL MMP budget (activity 2b).

Equipment

EURL MMP needs to purchase a new flow cytometer, only dedicated to SCC analyses, to perform the experiments required for the different phases of this project: feasibility study in 2015, and later certification study. This equipment will also be used for other EURL projects on SCC (including 1.1.1).

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 COW'S MILK

Duration: 2015 - expected end: 2020

Objective

EU Regulation (EC) n° 2074/2005 modified by Regulation (EC) n° 1664/2006 prescribes the International Standard EN ISO 11816-1, fluorimetric method for the determination of alkaline phosphatase (AP) activity in milk, as the reference method for own checks and official controls of this criterion in pasteurized milk. To date, this method is the most pertinent and reliable method available although it has the disadvantage of being tied to a single supplier in terms of material and reagents.

In 2013, an article has been published on "Fluorometric detection of active AP and GGT in fluid dairy products from multiple species" [*G. Ziobro (FDA, USA) and al. - Journal of Food Protection - Vol. 76, No. 5, 2013, Pages 892–898*]. The methodology cited for the determination of AP activity in milk is a fluorimetric detection using a microplate reader and

reagents readily available from most chemical suppliers, which is a key advantage over the method of EN ISO 11816-1.

Currently, when implementing the reference method of EN ISO 11816-1 which depends upon a unique manufacturer of material and reagents, laboratories are exclusively linked to their single local distributor. In certain countries, this limited market of material and reagents induces high costs and equipment maintenance not always efficient. This situation can quickly become problematic for the correct use of the reference method.

Therefore, the EURL intends to explore the possibility to use for official controls a method based on the one described in Ziobro's publication cited before, which is totally open in terms of material and reagents.

Expected output and time of delivery

The recently published protocol shares similarities with the one developed for the determination of gamma-glutamyl transferase in milk since it follows the same methodology. Therefore, based on our experience on the fluorimetric detection using a microplate reader, EURL will conduct preliminary work assays, in 2015, on cow's whole milk in order to evaluate and if needed to amend the recently published protocol.

Mission

Visit of the Swiss NRL (Bern, Switzerland, 2 days) which has already implemented the new method.

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

2.2.2 ALKALINE PHOSPHATASE ACTIVITY IN CREAM (MULTI-ANNUAL)

Duration: 2015 - expected end: 2020

Objective

During the last workshop (3-4 October 2013), the need of a legal limit for alkaline phosphatase (AP) in cream was highlighted. Indeed in 2012, the IE-NRL had raised the problem of a gap in legislation on AP limits for products other than milk and the need for separate limits for AP in cream and whey when they are pasteurized separately.

In June 2014, this issue has been addressed during the meeting of the Competent Authorities' 'WG Hygiene' and CAs welcomed the proposal to set a legal limit for AP in cream.

Expected output and time of delivery

In 2009, a EURL study on liquid cream demonstrated that sample preparation did not present any specific difficulties. However, during the 2013 workshop, Pr. Luisa PELLEGRINO (Italian expert) presented preliminary results of a study on AP determination in cream,

demonstrating the difficulties to achieve homogeneity of samples and the need to pursue this work.

In 2015, the EURL will conduct a bibliographic review related to sample preparation of cream. In addition as a preliminary step, EURL intends to perform in 2015 experiments on commercially available pasteurized and UHT cream samples to define the optimal sample preparation conditions for the determination of AP activity using the fluorimetric method (EN ISO 11816-1). Experiments will focus on the critical steps highlighted by the previous work of Pr. PELLEGRINO, that is to say: fat content, temperature and cream ageing.

Subsequently, in 2016 and beyond, EURL would initiate the validation of the method on determination of AP activity in cream; and the subsequent standardization of the method. EURL would also coordinate a European study aiming at generating data on the content of residual AP in pasteurized cream produced under different processes and heat treatments in the different EU Member States.

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

2.2.3 DEVELOPMENT OF A REFERENCE MATERIAL FOR AP

Duration: 2015-2017

Objective

During the IDF/ISO Analytical Week of 2014, the perspective of the production of a reference material (RM) for the 'ALP method in cheese' has been addressed. A preliminary work will be conducted by Dr Braun (MUVA, DE), it will focus on preparation of a freeze-dried raw milk cheese and follow up of its ALP activity evolution over time.

Expected output and time of delivery

EURL will carry out a survey on the NRLs' needs for reference materials for AP determination, and on NRLs' willingness to collaborate in their possible development.

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)

EURL MMP will organize in 2015 a 4th training session for the NRLs on SCC in raw cow's milk, with the reference method EN ISO 13366-1.

Training costs

In case of need, the travel and stay costs of 3 trainees at maximum would be covered (3 days).

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 2015 but EURL MMP will follow-up with any individual request from the NRLs.

Training costs

In case of need, the travel and stay costs of 3 trainees at maximum would be covered (3 days).

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: 2008-2015

Objective

The EURL MMP has been working 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 pasteurized cow's milk.

In this context, the EURL MMP has been steering over the last 4 years a European study aiming at generating data on the content of residual AP in pasteurised cow's milk cheese in the different EU Member States.

Expected output and time of delivery

The tentative limit of 10 mU of AP/g is globally acceptable in pasteurized cow's milk cheeses, according to a vast majority of the results submitted so far by the NRLs. However, this limit was not respected for some German (Tilsit) and French (Maroilles and Munster) cheeses which belong to the same type of cheese, namely washed rind cheeses (WRC).

In 2015, EURL MMP will further investigate the case of WRC then analyse the results obtained by NRLs, draft and distribute the final report of this study. At the annual workshop, EURL and NRLs will discuss a recommendation to DG SANCO for the setting-up of a limit for AP in pasteurized cow's milk cheeses.

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 STANDARDIZATION AND CERTIFICATION WORKS (MULTI-ANNUAL)

4.3.1 CRITICAL POINTS FOR IMPLEMENTATION OF THE REFERENCE METHODS FOR SOMATIC CELL COUNTING

Duration: start: 2015- expected end: 2016

Objective

At the 2014 workshop, NRLs highlighted the need to draft a check-list of critical points for the implementation of the reference method, EN ISO 13366-1. It was agreed that such a practical document would be useful, in addition to the Standard, since this is a microscopic method. It was expected to improve the implementation by NRLs, and other laboratories at national level, of this method.

Expected output and time of delivery

In 2015, EURL will prepare a first proposal for this document, which would then be circulated to NRLs to have their input and prepare a second draft.

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

4.3.2 PARTICIPATION TO ISO/IDF STANDARDIZATION AND CERTIFICATION WORKS (MULTI-ANNUAL)

On behalf of EURL MMP, participation to:

1. The IDF/ISO standardization works, and related certification 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, in particular:
        - Launching of validation studies by the certification body MicroVal 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 (V2, 21/01/2013)
    - Total flora: alternative methods, conversion relationship, in particular:
        - Revision of EN ISO 21187/IDF 196, which provides guidance for establishing and verifying a conversion relationship between routine method and reference method;
        - Conduction and outcome of validation studies by MicroVal of the instrumental methods for TF in raw milk.
    - Determination of alkaline phosphatase and other pasteurisation tracers in milk and milk products: reference and alternative methods,
    - Statistical & sampling aspects.

2. The 2015 IDF/ISO Analytical Week (Namur, Belgium, April 2015) and the meetings of the groups dealing with the topics mentioned above
    - Mission of 3 EURL representatives:
        - Rabeb MILED for groups on somatic cells count and total flora (conversion relationship),
        - Hanène GHEZZAL 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