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French agency for food, environmental and occupational health safety

Maisons-Alfort laboratory for food safety

EU-RL MMP
European Union Reference Laboratory for Milk and Milk Products

2011 Work Programme of the Reference Laboratory of the European Union for Milk and Milk Products

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French agency for food, environmental and occupational health safety – Maisons-Alfort laboratory for food safety

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The Maisons-Alfort Laboratory for food safety of Anses (French agency for food, environmental and occupational health safety) –formerly Afssa-Lerqap- foresees to undertake, as European Union Reference Laboratory (EU-RL) for milk & milk products (EU-RL MMP, formerly CRL MMP), the following works in 2011 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:
    - 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 EU-RL foresees in particular to provide 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:
    - 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: in brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2011), 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.

1.1.1. STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY TRIALS ON TOTAL FLORA IN RAW GOAT'S MILK

(multi-annual)

The EU-RL (Unit HMPA) will complete in 2011 an investigation study (stability and homogeneity) to find a way, such as the addition of a chemical agent, to stabilize sufficiently the total flora (TF) contamination of raw goat's milk, in order to organize PT trials on TF enumeration in raw goat's milk samples. It is intended to select a formula adapted to TF; formula which would allow the bacteria to grow on plates after the dilution steps.

1.1.2. STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY TRIALS ON SOMATIC CELLS IN RAW COW'S MILK

(multi-annual)

The EU-RL (Unit HMPA) will conduct in 2011-2012 an investigation study (stability and homogeneity) to find a way, such as the addition of a chemical agent, to stabilize sufficiently the somatic cells contamination of raw cow's milk, in order to organize PT trials on somatic cells count in raw cow's milk samples.

1.1.3. ENUMERATION OF TOTAL FLORA AT 30°C IN RAW GOAT'S MILK

(annual)

The EU-RL (Unit HMPA) will organize in 2011 an inter-laboratory trial on the total flora enumeration at 30°C in raw goat's milk by the reference method, the Standard EN ISO 4833 (total plate count method).

1.2. ANALYTICAL DEVELOPMENT

(multi-annual)

1.2.1. DETERMINATION OF TOTAL FLORA AT 30°C AND SOMATIC CELLS IN RAW MILK BY AN INSTRUMENTAL METHOD

The EU-RL (Unit HMPA) intends to complete in 2011 its experimental study for raw cow's and goat's milk, using a flow cytometer (Bactocount) purchased in 2007, as an alternative method to the bacterial total flora (TF) count and to the somatic cells count (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 factor (variation in breeds, period of lactation, type of feeding,..).

For that purpose, batches of raw cow's and goat's milk delivered at regular intervals of time will be analysed in parallel by the reference methods and by the Bactocount for TF and SCC.

Moreover, the EU-RL (Unit HMPA) will launch in 2011 a study to characterize the microbial composition of raw milks by PCR-DGGE (Denaturing Gradient Gel Electrophoresis).

1.2.2. 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.

In 2010, the EU-RL will have conducted an enquiry to the NRLs to collect existing national hygienic requirements/microbiological criteria on cows' colostrum. The synthesis of the enquiry will be prepared in 2011.

1.3. COORDINATION OF THE NRL ON DETERMINATION OF TOTAL FLORA

(multi-annual)

Since the publication of the Standard EN ISO 21187 on the conversion factors between the routine method and the reference method for TF determination, the EU-RL has been supervising the NRLs on how they coordinate the implementation of the Standard by the network of laboratories in charge of routine control of raw milk. In particular, all conversion factors should be recalculated according to the Standard in each Member State and it is intended to have only one conversion factor per country.

This topic will be covered at the 2010 annual workshop of the NRLs which will be dedicated to TF in raw milk (30 September/1 October), and actions to be conducted in the future, in particular in 2011, will be defined at that workshop.

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

(multi-annual)

IDF/ISO is conducting a revision of the Standard IDF 161 detailing the validation protocol of a routine method against a reference method for the TF determination in raw milk.

The EU-RL will follow this standardization work and will ensure a liaison with the works undertaken as EU-RL with the NRLs network.

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

(multi-annual)

Given the deficiencies of the reference microscopic method for SCC in raw milk (lack of reproducibility) and the limited number of laboratories using it, it is of utmost importance to develop Certified Reference Materials, which are not currently available.

In 2011, the EU-RL intends to bring its support to identify the possibilities to develop certified reference materials by the JRC/IRMM in Geel. For that purpose, DG SANCO is to contact JRC to organize a meeting.

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

(multi-annual)

IDF/ISO has initiated the setting-up of a reference system for SCC in raw milk, given the deficiencies of the microscopic reference method to provide reference values comparable between different laboratories. It is intended that this reference system, in addition to the reference method, would take account of reference materials and of instrumental methods used in routine. A network of expert laboratories is intended to be settled, to define assigned values associated to reference materials used for the calibration of instrumental methods.

The EU-RL will go its participation to the IDF/ICAR working group in charge of developing this reference system as to envisage how it could be beneficial to the CRL/NRLs own works, to implement the requirements of Regulation 853/2004 concerning SCC.

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.

2.1. INTERNATIONAL VALIDATION TRIAL AND, IN PARALLEL, INTER-LABORATORY PROFICIENCY TESTING FOR THE NRL

(annual)

The EU-RL (Team CAT-AP) will organize in 2011 an international validation study on the determination of alkaline phosphatase activity in cheese by the fluorimetric method, in order to generate precision data according to ISO 5725-2.

NRLS will be asked to participate to the study. The set of data submitted by NRLs will be extracted from the total data and submitted to a distinct statistical evaluation as an interlaboratory assessment trial.

2.2. ANALYTICAL DEVELOPMENT

(multi-annual)

In 2011, the EU-RL (Team CAT-AP) intends to conduct the following activities.

2.2.1. STUDY OF THE IMPACT OF THE BETWEEN-INSTRUMENTS VARIABILITY TO THE OVERALL VARIATION OF RESULTS OBTAINED BY DIFFERENT FLUOROPHOS UNITS

(multi-annual)

The EU-RL works with three different Fluorophos instruments. It is deemed important to evaluate the impact of the instruments themselves on the variation of the results obtained on the same test samples, when analysed using the same reagents and measured on the three instruments available.

In 2011, the EU-RL will launch a study on different milk and cheese samples. The EU-RL will share the conclusions of the study with NRLs and also with the instrument manufacturer.

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

The EU-RL will continue the study of AP levels for species other than cow's milk. The purpose of this work is to support DG SANCO in prescribing legal limits of AP activity in milk from different species.

EWE'S MILK

In 2011, the EU-RL will work on the establishment of phosphatase 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.

Once the pasteurisation conditions for ewe's milk will have been identified, the EU-RL intends to start work on raw and laboratory-pasteurised milk to initiate the project aiming to the setting up of AP limits in ewe's pasteurised milk.

CAMEL'S MILK

A project to characterize the heat-treatment of camel's milk is developed within a technical collaboration between the Central Veterinary Research Laboratory (CVRL) of Dubai and the German NRL.

The EU-RL is closely involved in this project at the request of DG SANCO: experimental work is to start only upon endorsement of the work program by EU-RL, the EU-RL is to be advised regularly on progress of the work and will scrutinize results obtained and conclusions drawn before their adoption.

2.2.3. DETERMINATION OF ALKALINE PHOSPHATASE IN CHEESES

REVISION OF THE INTERNATIONAL STANDARD ISO 11816-2/IDF 155-2

The EU-RL will continue acting as Project Leader for this topic and will progress the item during all stages prescribed within the official procedures of standardisation. It is expected that in 2011 the text will get a positive vote to move to the Committee Draft stage.

Technical comments may still be forwarded at this stage of voting and this may result to some minor technical changes after the EU-RL has studied the amendments proposed.

COORDINATION OF THE SURVEYS CONDUCTED BY NRL ON CHEESE MADE FROM PASTEURISED COW'S MILK.

In 2011, the EU-RL will continue the coordination of the experiments performed by NRLs on soft, hard and semi-hard cheeses made from cow's pasteurised milk.

This European study aims to collect information about the content of residual AP in pasteurised cow's cheese so as to set up legal limits of AP allowing for the distinction between cheeses made from pasteurised cow milk and cheeses made from cow non-pasteurised milk.

PARTICIPATION TO THE STUDY OF AP CONTENT IN PASTEURIZED COW'S CHEESE

The EU-RL will participate to the above mentioned survey, particularly when difficult cases are raised by NRLs (for ex. AP content in French pasteurised Munster cheese).

2.2.4. COMPARISON OF THE CHEMILUMINESCENT/FLUORIMETRIC METHODS

In 2011, the EU-RL intends to put into practice the approach of the accuracy profile (acceptability limits) to assess the equivalence of the chemiluminescent method versus the official fluorimetric method. Comparison will deal with cow's skim and semi-skim milk and, if possible, with goat's whole milk.

2.2.5. HEAT TRACERS OTHER THAN AP, DEVELOPMENT OF ANALYTICAL PROTOCOLS

The EU-RL will participate to an international project (Project leader: Dr George Ziobro, US/FDA) aiming to identify other pasteurisation tracers when AP is inappropriate, and to collect information on relevant analytical methods. Depending on the progress of the work item, preliminary assays on the identified protocols may be conducted in 2011.

2.2.6. REACTIVATED PHOSPHATASE

No experimental work is on the work programme but the EU-RL will continue to keep an eye on the topic. In case FDA can accept the suggestion, an exchange of views with scientific experts of the US/FDA competent laboratory is envisaged.

3. ASSISTANCE TO THE NRL

Upon requests of NRLs, the EU-RL may receive NRL staff for individual training on specific topics.

4. NRLS WORKSHOP

The EU-RL will organise in 2011 the 14th NRLs Workshop of general scope. In particular, this workshop will enable:

1. to make a point of works undertaken by the EU-RL since the last general workshop of 2009, in particular further to the 2010 Workshop dedicated to Total Flora;
2. to envisage the work programme for the following years.

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 US/UE negociations on veterinary agreement (dairy hygiene, total flora, somatic cell count and phosphatase activity),
- And any other question which may arise during the year.

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 raw milk:
   - somatic cells count: reference and alternative methods,
   - total flora: alternative methods,
   - phosphatase test: reference and alternative methods, and other pasteurisation tracers.
2. The 2011 IDF/ISO Analytical Week (Lyon, France, May 2011) and the meetings of the groups dealing with the topics mentioned above.