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French agency for food, environmental and occupational health safety

Maisons-Alfort laboratory for food safety

EURL CPS
European Union Reference Laboratory for Coagulase Positive Staphylococci

2013 Work Programme of the European Union Reference Laboratory for Coagulase Positive Staphylococci

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French Agency for Food, Environmental and Occupational Health Safety – Maisons-Alfort Laboratory for Food Safety
23, avenue du Général de Gaulle - F94706 Maisons-Alfort Cedex - Telephone: + 33 149 771 300 - Fax: + 33 143 689 762 - www.anses.fr

INTRODUCTION

In May 2006, the Maisons-Alfort Laboratory for Food Safety of Anses (French agency for food, environmental and occupational health safety) has been nominated European Union Reference Laboratory for Coagulase Positive Staphylococci (EURL CPS), including *Staphylococcus aureus* and their toxins (see Regulation 776/2006).

The EURL CPS foresees to undertake the following actions in 2013, according to the actions planned at the 6th Workshop of the National Reference Laboratories (NRLs) (23-25 May 2012).

Most of these activities aim at implementing, from an analytical point of view, the EC Regulation 2073/2005 on microbiological criteria for foodstuffs, modified by the Regulation 1441/2007, which includes in particular:

- 5 process hygiene criteria on CPS, defining a quantitative limit in:
    - cheeses made from raw milk or from heat-treated milk, ripened cheeses, and unripened soft cheeses,
    - milk/whey powder,
    - cooked crustaceans and molluscan shellfish.
- 1 food safety criterion on staphylococcal enterotoxins (SETs), requiring absence in 25 g in cheeses, milk/whey powder, to be tested when CPS enumeration is higher than 10^5 cfu/g when testing the above mentioned criteria on CPS.

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).

NB2: The activities are gathered according to the tasks allocated to EURLs, defined in 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 (MUTI-ANNUAL)

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

In particular, the EURL deputy manager has undertaken a visit of the NRLs, in order to better know them, their teams, premises, and exchange on their NRL missions and activities, as well as on their expectations from the EURL.
Mission in 2013: 1 visit to closely located 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 the 7th Workshop of the NRLs in 2013, of general scope:

- to make a progress report on works undertaken by the EURL and the NRL network since the 2012 Workshop;
- to envisage the work programme for 2014 and later.

This workshop will take place in Maisons-Alfort, France. Three experts would be invited, as well as NRLs from accessing countries.

0.3 SCIENTIFIC MONITORING AND COMMUNICATION (MUTI-ANNUAL)

The EURL teams will review scientific publications in the EURL area of competence, as well as communicate on the works conducted as EURL Lm, disseminate the outcome of works in the international scientific community (drafting of written publications, oral presentations and posters to international symposia).

1 DISPATCH OF METHODS AND PROFICIENCY TESTING TRIALS

1.1 DETECTION/ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI IN FOOD

1.1.1 STUDY OF SAMPLE TYPES USED FOR INTER-LABORATORY TRIALS (MULTI-ANNUAL)

Duration: start: 2011- expected end: 2013

Objective

The EURL CPS (Unit EDB) is studying several food matrices to be used as samples for proficiency testing (PT) trials on CPS enumeration, as to cover the food types concerned by microbiological criteria. Indeed, NRL ability to implement the reference methods for CPS enumeration (EN ISO 6888-1, 2) may vary, depending on the type of food matrices analysed (sample preparation). Up to now, the EURL has investigated the following sample types: pasteurized milk, fresh dairy product (cheese), dried food (milk powder). The next matrix to be investigated is a cooked crustacean or molluscan shellfish.

This project comprises two stages:

- in 2011, a fist study has been conducted with artificial contamination of each sample, of test portion size, to be directly analysed by NRLs;

- from 2012, a second study is being conducted as to the NRLs have to sub-sample themselves the test portion (important initial step of the analyse): the EURL performs a global artificial contamination of all samples (at one contamination level) which are distributed in 13 g size samples for each participant.

Expected output and time of delivery

In 2013, the EURL will optimize and complete this second study, at different contamination levels and using, if necessary, a bacteriostatic agent.

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

1.1.2 COMPARISON OF VARIOUS INOCULATION TECHNIQUES OF SOLID FOOD MATRICES FOR PT TRIALS (MULTI-ANNUAL)

Duration: started: 2011 – expected end: 2014

Objective

Solid food matrices are part of foodstuffs included in the microbiological criteria for CPS, in EC Regulation 2073/2005, thus it is necessary to organize PT trials on solid food matrices. The EURL CPS (Unit EDB) has already organized PT trials on such sample types, but the artificial contamination technique should be optimized, as to be easily implemented by the

EURL and the NRLs, to be repeatable as well as ensuring a satisfactory homogeneity and stability of the contamination.

In addition, the EURL has organized PT trials up to now without including sub-sampling of test portion: the possibility to include this initial step of the analysis, which can have a major impact on the validity of the analyses of solid matrices, will be tested.

Expected output and time of delivery

In 2013, the EURL MMP (Unit EDB), in collaboration with the EURL *Listeria monocytogenes*, will conduct a bibliographic review and launch an enquiry to the NRLs to collect their experience as PT trial organisers at national level.

The EURL will also test and compare different inoculation techniques of solid food matrices (in 2013 decorticated crustaceans such as prawns, mussels), in-depth or in surface, so as to optimize the combination between the solid food matrix and the inoculation technique. In particular, the EURL will study the homogeneity and stability of these newly developed sample types.

If satisfactory results would be obtained, this study would be used by the EURL CPS for future PT trials and could help NRLs for the organization of their inter-laboratory PT trials at national level.

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

1.1.3 PT TRIAL

Objective
The inter-laboratory PT trials organised by the EURL for the NRLs aim at evaluating the ability of the NRLs to apply satisfactory the reference methods EN ISO 6888-1&2 prescribed by Regulation 2073/2005for CPS enumeration in food.

Expected output and time of delivery
In 2013, the EURL CPS (Unit EDB) will organize an inter-laboratory PT trial on the CPS enumeration by one of the reference methods EN ISO 6888-1 or 2, using dried milk powder or pasteurized milk as a matrix.

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

1.2 DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

1.2.1 EUROPEAN SCREENING METHOD (MULTI-ANNUAL)

Duration: started: 2010 – expected end: 2013

Objective

The European screening method of the EURL CPS (ESM) is cited as reference method in the criterion 1.21 for staphylococcal enterotoxins (SEs) in cheeses, milk/whey powder, by the EC Regulation 2073/2005 modified (Annex I, Chapter 1). This method is used for own checks and official controls in the EU Member States.

This method includes an initial step of SE extraction/concentration by dialysis concentration, followed by a detection step. Currently, this detection step needs to be based on an immuno-enzymatic reaction, and it is not feasible to ask NRLs, official food control laboratories or own check laboratories to prepare in-house test kits. Thus the use of commercial ELISA kits is necessary for the detection step in routine analyses for own checks or official controls.

To provide practical guidance to the NRLs, official control laboratories and indirectly own check laboratories, the EURL CPS (Team CAT-BAC) compares the performance of the available kits and to recommend the use of the satisfactory ones in the frame of ESM. The possibility to use the Ridascreen SET Total (R-Biopharm) is currently investigated.

The EURL CPS will maintain ESM and update it until the method for SE detection in food will be standardized by CEN and ISO. When the Standard method will be published, it is intended to withdraw ESM. Validation studies on the method to be standardized, within the frame of the CEN Mandate M/381, will be complementary to the activities undertaken as EURL CPS, in terms of matrices covered and commercial kits studied.

Expected output and time of delivery

Once the results of the 2nd method validation interlaboratory study, to be organized by R-Biopharm (manufacturer of Ridascreen SET total) under EURL supervision, would be made available to the EURL and if the EURL would consider the outcome satisfactory, the EURL would prepare in 2013 a new version of the ESM leaving the choice, for the detection step, between Vidas SET 2 (bioMérieux) and Ridascreen SET Total kits.

From now on, experimental works on this screening method won't be internally conducted in the frame of EURL activities, but in the CEN Mandate.

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

1.2.2 CONFIRMATORY ELISA METHOD (MULTI-ANNUAL)

Duration: started: 2008 – expected end: 2013

Objective

Positive results obtained with the European Screening method used for own checks and official controls (see 1.2.1) require to be confirmed. The confirmatory method for the identification and quantification of SE types (SEA to SEE) in food has been up to now an in-house ELISA-based technique developed by the EURL CPS, which could not be transferred to NRLs, by lack of sufficient availability of suitable antibodies.

The purpose of this work was to develop a confirmatory ELISA method which could be transferred to the NRLs, enabling them to confirm positive results obtained by official control laboratories in their respective countries, in particular in the frame of investigation of staphylococcal food poisoning outbreaks (SFPOs) or for official controls.

One mission is scheduled for an NRL which would need assistance for the on-site implementation of the method.

This method will be also required for the development of certified reference materials to assign a value.

Expected output and time of delivery

The EURL has completed in 2012 the development of the quantitative ELISA method for SEA to SEE types.

In 2013, the EURL CPS (Team CAT-BAC) will go on transferring this method to the NRLs, after their training by the EURL (see 3.1.2).

Once trained and the method transferred to them, the NRLs can give themselves a fast answer (1-day analysis), instead of requesting confirmatory analyses to the EURL.

Moreover, in 2013, the EURL will continue to test different batches of the reagents (antibodies against SEA to SEE) necessary for the implementation of the quantitative ELISA method for the NRLs.

Upon request and for the NRLs which have not been trained to the confirmatory method, the EURL will also provide scientific and technical assistance to DG SANCO and to the NRLs, especially to perform confirmation analysis of positive results obtained by the NRLs with the ESM, in the frame of official controls performed according to the Regulation 2073/2005 modified.

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

1.2.3 PT TRIAL

Objective
The inter-laboratory PT trials organised by the EURL for the NRLs aim at evaluating the ability of the NRLs to apply satisfactory the EURL CPS European Screening Method (ESM) prescribed by EC Regulation 2073/2005for SE detection in milk products.

Expected output and time of delivery
The EURL CPS (Team CAT-BAC) will organize a PT trial in 2013 on SE detection in food, using the applicable ESM version.

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

1.3 CPS STRAIN CHARACTERIZATION AND TYPING

1.3.1 DISPATCH OF STRAINS (MULTI-ANNUAL)

Upon request of the NRLs, the EURL CPS (Unit CEB) would send them CPS field strains from its collection. Transportation of strains to the NRLs will be sub-contracted.

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

1.3.2 ANALYSES FOR NRLS

Objective

In the frame of their involvement in the investigation of national staphylococcal food-poisoning outbreaks, NRLs CPS may need to confirm the food implicated by the detection of *se* genes in CPS strains, PFGE or spa-typing, in addition to the SE detection in the same food. Several NRLs have not the capacity to perform these methods, thus their need to request the EURL to do this analysis.

Expected output and time of delivery

In 2012, the EURL CPS (Unit CEB) has performed *se* gene detection of 16 strains from 2 NRLs. The EURL has also performed the PFGE characterisation of 29 strains from 1 NRL.

Upon request of the NRLs, the EURL CPS (Unit CEB) will perform in 2013 the detection of *se* genes by conventional PCR or the typing of CPS strains sent by NRLs.

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

1.3.3 EUROPEAN STRAIN COLLECTION (MULTI-ANNUAL)

Objective

As agreed at the 2011 & 2012 Workshops, the EURL (Unit CEB) may settle a European collection of CPS strains in the following years, at the condition that DG SANCO approves it. This collection could be used for method validation and for SFPO investigation, at national or European levels.

In 2013 and for preparing the discussions at the 2013 Workshop, the EURL (Unit CEB, in collaboration with the Team CAT-BAC) would draft and dispatch the outcome of a questionnaire to be sent in 2012 to the NRLs, in order to identify their needs for a European CPS strain collection and to evaluate their capacity for strain supply, the diversity of strain origin, etc.

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

2 ANALYTICAL DEVELOPMENT

2.1 DETECTION/ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI IN FOOD

2.1.1 MEASUREMENT UNCERTAINTY: IMPACT OF SUB-SAMPLING OF THE TEST PORTIONS (MULTI-ANNUAL)

Duration: started: 2010 – expected end: 2014

Objective

To conduct analyses for own checks and official controls related to the quantitative criteria on CPS defined in EC Regulation 2073/2005 modified (criteria 2.2.3, 2.2.4, 2.2.5, 2.2.7 & 2.4.1 in Annex I, Chapter 2), it is important to know and to control the measurement uncertainty (MU) associated to the analytical results. For example, the analytical result found may comply with the limit settled in the microbiological criterion whereas the true result (lying in the uncertainty range) may not comply: in that case, a wrong interpretation of the result may be taken if ignoring MU. A correct interpretation of analytical results, in terms of conformity with regulatory limits, thus requires the knowledge of MU associated to these results as well as the limitation of this uncertainty as far possible.

In the series of Standards EN ISO 6887-2 to 5 on the preparation of test samples for microbiological analyses, it is not specified how to sub-sample the test portion in the laboratory sample (sample that is sent to the laboratory), depending on the different types of food matrices to be submitted to microbiological analyses. This stage is however recognized as a major source of MU, in particular for solid matrices characterized by heterogeneous bacterial contaminations, such as matured cheeses.

The purpose of this study is to harmonize the procedure of sub-sampling the test portion in solid matrices, such as cheeses, thus (i) reducing the overall MU, and (ii) better ensuring that the contamination of a sample is correctly reflected in the test portion taken and analyzed.

The outcome of this study would be transferred to ISO (i) to provide data for the revision of ISO/TS 19036 (MU estimation for quantitative determinations), to quantify the MU part liked to sub-sampling of test portions, for solid matrices, and (ii) to revise EN ISO 6887 series to better define the procedure of sub-sampling the test portion in solid matrices.

Expected output and time of delivery

In 2013, the EURL CPS (Unit EDB, in collaboration with the Team CAT-BAC) will complete an investigation study launched in 2011 on the heterogeneity of CPS contamination in cheese samples.

For different types of cheeses (soft uncooked paste –Bleu de Gex, and pressed uncooked paste –Tome au marc et Morbier), the experimental study on sub-sampling the test portion will be continued with conventional and molecular techniques for CPS quantification, in

order to assess the impact of sub-sampling on the measurement uncertainty and on the representativeness of the analytical results. This study requires the analysis of naturally contaminated samples from various origins, to be provided in particular by NRLs. The transportation of samples from NRLs to the EURL will be sub-contracted.

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

2.2 DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

2.2.1 EVALUATION QUANTITATIVE ELISA WITH SEG AND SEI

Duration: started: 2012 – expected end: 2013

Objective

Staphylococcal food-borne outbreaks involving CPS strains encoding for toxin types SEG and SEI have been reported in Europe. Currently, no detection method is available to detect and quantify these SE types in food samples.

The scope enlargement of the quantitative ELISA method for the detection/quantification, to cover the SEG and SEI types, would enable to better implement the microbiological criterion of EC Regulation 2073/2005 (SE not detected in 25 g test portion of cheese, milk and whey powders), with the purpose of ensuring a high level of consumers' protection.

Expected output and time of delivery

In 2012, the EURL has chosen two antibodies against these types of toxins (SEG and SEI) and has started the optimization conditions of the method, as well as the construction of the ELISA systems.

In 2013, the EURL will carry on the optimization of the method: antibodies concentrations, standard curves, determination of limit of detection and of limit of quantification and cross reactions.

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

2.2.2 DEVELOPMENT OF CRM IN COLLABORATION WITH JRC/IRMM (MULTI-ANNUAL)

Duration: started: 2011 – expected end: 2014

Objective

The need of certified reference materials (CRMs) for SEs in food is one of the priorities of the EURL CPS and a major need for the NRLs. This need has been also acknowledged by a letter from DG SANCO to JRC/IRMM (Geel, BE), with the collaboration of the EURL CPS.

JRC/IRMM, in collaboration with the EURL CPS (Team CAT-BAC), has started in 2011 the project to develop CRMs on SE in a lyophilized cheese matrix.

Expected output and time of delivery

The first feasibility study has been carried out in 2012.

The actions planned in 2013 are the following:

- to train volunteering NRLs for SE analysis by the EURL CPS quantitative ELISA method (see 1.2.2);
- to conduct an enquiry to the NRLs, as to investigate their potential need of CRM, the different types of use, as well as the potential use at national level by official control laboratories, as to estimate the CRM batch size according to the needs;
- to investigate other needs, such as other combinations (food type, SE types).

Mission: 2 persons for 1 meeting at JRC/IRMM, Geel, BE.

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

2.3 CHARACTERIZATION AND TYPING OF STRAINS, EPIDEMIOSURVEILLANCE

2.3.1 DEVELOPMENT OF SE GENES DETECTION BY MULTIPLEX PCR (MULTI-ANNUAL)

Duration: started: 2011 – expected end: 2013

Objective

For the investigation of staphylococcal food-poisoning outbreaks, it is often necessary to confirm the food implicated by the detection of *se* genes in CPS strains, in addition to the SE detection in the same food.

The EURL CPS (Unit CEB) has been developing a multiplex real-time PCR scheme for the detection of 13 *se* genes. The multiplex assay for *seg-sei-sej* genes demonstrated good efficiency and good sensibility on plasmids.

Expected output and time of delivery

In 2013, the EURL will test the efficiency and sensitivity of the multiplex RT PCR assays for *seb-sec-sed* genes and *sea-see-seh* genes, which will require subcontracting for sequencing. Then, the EURL will launch a validation study of these assays on a large collection of food *S. aureus* field strains. The data will be compared with those obtained by conventional PCR. The NRLs were invited to take part to this validation study, on a common panel of strains: the UK-NRL (HPA) and the NL-NRL (NVWA) already agreed to participate.

Mission: 1 visit of a collaborating NRL.

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

3 NRL TRAINING

3.1 DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

3.1.1 EUROPEAN SCREENING METHOD

In 2013, the EURL CPS (Team CAT-BAC) intends to organize for NRLs one (or several) training session(s) on SE detection according to the EURL CPS European Screening Method (ESM), depending on the needs and in particular if the ESM is updated (see 1.2.1).

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

3.1.2 QUANTITATIVE ELISA METHOD

In 2013, the EURL CPS (Team CAT-BAC) intends to organise for NRLs 2 or 3 training sessions on SE confirmation according to the EURL CPS quantitative ELISA method (see 1.2.2), depending on the needs.

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

3.2 CPS STRAIN CHARACTERIZATION AND TYPING

In2013, the EURL CPS (Unit CEB) will organize a training session dedicated to sub-typing of *S. aureus* strains by PFGE. This 2-day session will include technical and theoretical courses and will take place at the EURL laboratory either at end of January 2013 or in October 2013.

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

4 TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION

4.1 DG SANCO ACTIVITIES (MULTI-ANNUAL)

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

- If needed, participation of the EURL CPS, for the analytical aspects, to the update of Regulation 2073/2005 on microbiological criteria related to CPS and SEs
  Missions: 2 meetings in Brussels;
- and any new question which may arise during the year.

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

4.2 PARTICIPATION TO CEN/ISO STANDARDIZATION ACTIVITIES (MULTI-ANNUAL)

On behalf of the EURL CPS and as EC representative:

- Follow-up by the EURL CPS manager (Bertrand LOMBARD) of the activities of ISO/TC 34/SC 9[1] & CEN/TC 275/WG 6[2] for general aspects related to the standardization of reference methods in food microbiology, which concern in particular CPS and SE analysis (1 jointed plenary meeting –budget EURL *Listeria monocytogenes*);
- In particular, participation of the EURL CPS manager (Bertrand LOMBARD) to the works of two working groups of ISO/TC 34/SC 9 of specific interest for the EURL activities and for DG SANCO: WG 2 Statistics (1 meeting) and WG 3 "Method Validation"
  Missions: 1 meeting of WG 2 (NL) and 2 meetings of WG 3 (Milano, IT and TBD).
- Leadership by a EURL CPS scientist (Alexandra CAUQUIL) of the WG 13 of ISO/TC 34/SC 9, in charge of preparing an amendment to EN ISO 6888-1 (CPS enumeration, Baird Parker agar), to include an optional confirmatory test
  (no meeting outside Paris area).
- Leadership by a EURL CPS scientist (Jacques-Antoine HENNEKINNE) of the CEN TAG on SE detection, Project Leader for the validation of the method to be standardized
  (costs covered by CEN Mandate M/381, no cost in the EURL CPS budget).

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

[1] Sub-Committee 9 « Microbiology » of Technical Committee 34 « Food products »

[2] Working Group 6 « Microbial Contaminants » of Technical Committee 275 « Food analysis – Horizontal methods »