



EURL CPS

European Union Reference Laboratory for
Coagulase Positive Staphylococci

Maisons-Alfort laboratory for
food safety

2012 Work Programme of the European Union Reference Laboratory for Coagulase Positive Staphylococci

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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 2012, according to the actions planned at the 5th Workshop of the National Reference Laboratories (NRLs) (16&17 June 2011).

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: In brackets under each item, the scheduled duration of the action is indicated: either annual (limited to 2012), either multi-annual (on-going programme on several years).

0 GENERAL ASPECTS

0.1 GENERAL COORDINATION (EURL MANAGEMENT TEAM, PAFT DEPARTMENT) (MUTI-ANNUAL)

General coordination 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 EURL units.

0.2 WORKSHOP OF THE NRLS (ANNUAL)

The EURL will organise the 6th Workshop of the NRLs in 2012, of general scope:

- to make a progress report on works undertaken by the EURL since the 2011 Workshop;
- to envisage the work programme for 2013 and further.

This workshop will be hosted by the NRL of Malta, in La Valetta.

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 CPS, disseminate the outcome of works in the international scientific community (drafting of written publications, oral presentations and posters to international symposia).

1 DETECTION/ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI IN FOOD

Frame: The Standard methods EN ISO 6888-1 or 2 are cited as reference methods in the quantitative criteria of EC Regulation 2073/2005 for CPS.

1.1 PROFICIENCY TESTING TRIALS

1.1.1 INTER-LABORATORY PT TRIAL FOR THE NRLS (ANNUAL)

The inter-laboratory PT trials organised by the EURL for the NRLs aim at evaluating the ability of the NRLs to apply satisfactorily the methods for the analyses performed in the frame of controls prescribed by Regulation 2073/2005.

In 2012, the EURL CPS (Unit EDB) will organize an inter-laboratory trial on the CPS enumeration by one of the reference methods EN ISO 6888-1 or 2, using dried milk powder as a matrix. An investigation of the homogeneity and stability of this matrix type has been previously performed in 2009-2011.

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

As to be able to organize an inter-laboratory proficiency testing (PT) trial on a new matrix compared to the former PT trials, the EURL CPS (Unit EDB) will conduct an investigation study (stability and homogeneity) to prepare artificially contaminated cooked crustaceans and molluscan shellfish which could be used to prepare and dispatch samples for PT trials on CPS enumeration.

1.2 ANALYTICAL DEVELOPMENT

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

Context: 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 associated to the analytical results. For example, the 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 the measurement uncertainty. A correct interpretation of analytical results, in terms of conformity with regulatory limits, thus requires the knowledge of measurement uncertainty 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 measurement uncertainty, 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 sample in solid matrices, such as cheeses, thus (i) reducing the overall measurement uncertainty, and (ii) better ensuring that the contamination of a sample is correctly reflected in the test portion taken and analyzed.

In May 2011, the outcome of an enquiry about sub-sampling of cheese test portions was sent to the NRLs, allowing the acquisition of an inventory of practices among the NRLs/Member States.

In 2012, the EURL CPS (Unit EDB) will complete an investigation study about the heterogeneity of cheese samples in terms of CPS contamination. For heterogeneous cheeses, an experimental study on sub-sampling of the cheese test portions will be launched, in order to assess the impact of sub-sampling on the measurement uncertainty and on the representativeness of the analytical results. This would require the analysis of naturally contaminated samples from various origins. It is intended to conduct this study in collaboration with some NRLs. The transportation of samples from NRLs to the EURL will be sub-contracted.

1.2.2 ALTERNATIVE ENUMERATION METHODS OF COAGULASE POSITIVE STAPHYLOCOCCI IN FOOD (MULTI-ANNUAL)

Frame: Several NRLs have underlined that the Standard methods for CPS enumeration in food, EN ISO 6888-parts 1&2, cited as reference methods in EC Regulation 2073/2005, are quite heavy to implement in routine use, for large scale own checks or official controls: these methods indeed rely on conventional microbiology and part 1 includes a laborious confirmation step. The NRLs wished to be able to use, for these purposes, alternative methods, in particular PCR methods. It was then agreed that the EURL would investigate the different types of alternative methods that may be used, focusing on PCR methods.

Following the bibliographical study on rapid alternative methods for CPS enumeration, presented at the 5th workshop in 2011, the EURL CPS (Unit EDB) will start the implementation and the evaluation of real-time quantitative PCR methods specific for CPS. These techniques will be examined for their sensitivity, specificity and efficiency; their potential for quantification of CPS will be tested with artificially and naturally contaminated food products.

The implementation of these methods will require subcontracting for the manufacture of nucleic probes.

2 DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

2.1 OPTIMIZATION OF THE EUROPEAN SCREENING METHOD (MULTI-ANNUAL)

Frame: The European screening method of the EURL CPS (ESM) is cited as reference method in the criterion 1.21 for staphylococcal enterotoxins (SEs) 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. It includes an initial step of extraction/concentration of SEs 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 to prepare in-house test kits: the use of commercial ELISA kits is necessary for the detection step in routine analyses for own checks or official controls. To guide NRLs and official control laboratories, the EURL CPS (Team CAT-BAC) has to compare the performance of the available kits and recommends the use of the satisfactory ones in the frame of ESM.

Further to the lack of performance of the Transia Plate SET (Biocontrol System) kit, which was prescribed for the detection step in earlier versions of the ESM, the EURL CPS (Team CAT-BAC) has been evaluating the performance of other kits, Ridascreen SET Total (R-BIOPHARM), Vidas SET2 (bioMérieux), whose performances were estimated to be satisfactory based i) on data provided by the manufacturers, ii) on published data and iii) on data obtained in the EURL during performance testing. Intra- and inter-laboratory studies have been conducted on the methods using dialysis concentration and both kits for the analysis of SEs in milk and milk products on one hand, and in other food matrices on the other hand.

In 2012, as to complete the on-going validation study of the method using dialysis-concentration and the Ridascreen SET Total kit for the detection step of SEA to SEE in all types of food matrices, the manufacturer (R-BIOPHARM) will organize, under supervision of the EURL CPS (Team CAT-BAC), an additional interlaboratory study. The aim of this additional study is to obtain enough results statistically valid, according to the validation protocol of EN ISO 16140 (qualitative part). The manufacturer will send a report of the study to the EURL which will check it and draw a conclusion on the validation of the method using this kit.

Once this study completed, the ESM will be temporarily updated.

In a second step, the EURL, in collaboration with the network of NRLs, will launch the preparation of a new version of the ESM based on a different approach: to define for the detection step performance criteria which should be met by commercial kits, without mentioning specific kits.

2.2 PROFICIENCY TESTING TRIALS (ANNUAL)

The inter-laboratory proficiency testing (PT) trials organised by the EURL for the NRLs aim at evaluating the ability of the NRLs to apply satisfactorily the methods for the analyses performed in the frame of controls prescribed by Regulation 2073/2005 modified.

The EURL CPS (Team CAT-BAC) will organize at the end of 2011 / beginning of 2012 an inter-laboratory PT trial for SEA to SEE detection in cheese matrices and other types of food matrices with the updated version of the ESM.

Moreover, in case of satisfactory results of the additional inter-laboratory validation study of the method using dialysis concentration coupled to Ridascreen SET Total (see 2.1), a 2nd PT trial may be organized at the end of 2012, depending on the resources and time available.

2.3 DEVELOPMENT OF A CONFIRMATORY ELISA TEST FOR NRLS (MULTI-ANNUAL)

Frame: Positive results obtained with the European Screening method used for own checks and official controls (see 2.1) require to be confirmed. The confirmatory method for the identification and quantification of SE types SEA to SEE in food is currently based on an in-house ELISA-based technique developed by the EURL CPS, which cannot be transferred to NRLs, by lack of sufficient availability of suitable antibodies. The purpose of this work is to develop a confirmatory method which can be transferred to the NRLs, enabling them to confirm positive results obtained by official control laboratories in their respective countries.

In order to transfer to the NRLs a quantitative method, the EURL CPS (Team CAT-BAC) has been developing an ELISA test enabling to quantify SE types A to D (and/or E), based on the use of commercially available antibodies that NRLs could get from other sources than the EURL to design their own confirmatory method.

In 2012, following a recent staphylococcal food poisoning outbreak having involved CPS strains encoding for toxin types SEG and SEI, the EURL will test the performance of commercially available antibodies against SEG and SEH types in order to complete the development of a quantitative ELISA test in food.

2.4 USE OF MASS SPECTROMETRY FOR SE CHARACTERISATION AND QUANTIFICATION IN FOOD (MULTI-ANNUAL)

Frame: It has been noted in 2.3 that the current confirmatory method is ELISA-based. The situation where both screening and confirmatory methods are based on the same principle is not satisfactory, introducing a risk of bias in the confirmation of screening results. In order to avoid this risk, the EURL CPS (Team CAT-BAC) has been conducting in collaboration with CEA (French Alternative Energies and Atomic Energy Commission) a project to investigate an alternative tool to immunology, that is quantitative mass spectrometry (MS), to confirm and quantify SEs presence in food matrices.

From September 2011 to the end of 2012, the Protein Standards Absolute Quantification (PSAQs) for SEs (SEA, SEB, SEC, SED, SEE and SEG) will be transferred to the EURL CPS. At the condition the PSAQs are correctly transferred to us, the multiplex quantitative MS methodology using these PSAQs will be evaluated and characterized, with the following steps:

- 1st step: implementation of the method for one type of PSAQ in water condition (2011);
- 2nd step: implementation of the method for one type of PSAQ in spiked food matrices (2011-2012);
- 3rd step: implementation of the method for one type of PSAQ in naturally contaminated food matrices (2012);
- 4th step: implementation of the method for various PSAQs in food matrices (spiked and naturally contaminated samples) (2012-2013).

2.5 DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS (MULTI-ANNUAL)

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.

Following a meeting dated 15th June 2011 with JRC/IRMM (Geel, Belgium) at ANSES Maisons-Alfort, a project of CRM development based on cheese has been programmed by IRMM. The first steps of the project are planned from June 2011 to 2012.

The EURL CPS (Team CAT-BAC) will be closely associated to the development of these CRMs and will in particular evaluate the homogeneity and stability of the pilot batch to be prepared by IRMM.

2.6 EVALUATION OF SE DISTRIBUTION IN NATURALLY CONTAMINATED CHEESES (MULTI-ANNUAL)

The study presented in 1.2.1 will be also launched by the Team CAT-BAC, in collaboration with EDB Unit to study the SE distribution in naturally contaminated cheeses.

2.7 NRL TRAINING (MULTI-ANNUAL)

The EURL CPS (Team CAT-BAC) intends to organize for the NRLs in 2012 one (or two) training session(s) on SE detection by the updated European Screening Method. These training session(s) would mainly cover the first step of the method, the extraction by dialysis concentration, which requires specific technical skills.

Moreover and depending on the needs and available resources, other training sessions may be organized on the quantitative SEA to SEE ELISA detection method that would be transferred to NRLs (see 2.3).

2.8 TECHNICAL AND SCIENTIFIC ASSISTANCE TO DG SANCO AND NRLS (MULTI-ANNUAL)

Depending on the needs, the EURL CPS (Team CAT-BAC) will collaborate and 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.

3 CHARACTERIZATION AND TYPING OF STRAINS, EPIDEMIOLOGICAL SURVEILLANCE

Frame: In the DG SANCO support document to the call for the selection and designation of the new EURLs (SANCO/2214/2005), the Annex 1 describes the specific functions of the EURL CPS, which includes to keep abreast of developments in CPS epidemiology and to cooperate, as appropriate, with the Community structures involved into surveillance of CPS.

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.

3.2 DEVELOPMENT OF SE GENES DETECTION BY MULTIPLEX PCR AND OTHER MOLECULAR TECHNIQUES (MULTI-ANNUAL)

3.2.1 ANALYSES FOR NRLS

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

3.2.2 OTHER MOLECULAR TECHNIQUES

The EURL CPS (Unit CEB) is progressing in the development of a multiplex real-time PCR scheme for the detection of 13 *se* genes. In a first step, simplex PCR assays are developed. In a second step, triplex PCR assays will be tested. The specificity and sensitivity of the assay will be tested. Then, this assay will be tested on a large collection of food *S.aureus* field strains. The data will be compared with those obtained by conventional PCR.

The EURL CPS will also develop a Multi Locus Variable number tandem repeat Analysis (MLVA) protocol coupled with capillary electrophoresis. VNTRs loci already described will be tested on a large panel of strains including reference and field food strains. The obtained data will be compared with those obtained by PFGE. This study will be performed in close collaboration with the Orsay University (France) and CEERAM (France).

The implementation of these techniques will require subcontracting for gene sequencing of CPS strains.

3.3 PFGE TRAINING SESSION (MULTI-ANNUAL)

Upon NRL request, the EURL CPS (Unit CEB) may organize a training session on *se* genes detection by PCR, on PFGE sub-typing and *spa* typing of CPS. This training session may be coupled with the SE detection training session organized by the EURL CPS (Unit CAT-BAC) (see 2.9).

3.4 EUROPEAN STRAIN COLLECTION (MULTI-ANNUAL)

As agreed at the 2011 workshop, the EURL (Unit CEB) will establish a European collection of CPS strains during the next years.

In 2012 the EURL (Unit CEB, in collaboration with the Team CAT-BAC) will send a questionnaire to the NRLs, in order to evaluate their capacity for strain supply, the diversity of strain origin, etc. The outcome of the questionnaire will be presented at the 2012 workshop.

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 manager, for the analytical aspects, to the update of Regulation 2073/2005 on microbiological criteria related to CPS and SETs;
- and any new question which may arise during the year.

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 manager of the activities of ISO/TC 34/SC 9¹ & CEN/TC 275/WG 6² for aspects related to the standardization of reference methods for CPS and SETs (1 jointed plenary meeting –budget EURL *L. monocytogenes*).

In particular, participation to the works of one working group of ISO/TC 34/SC 9 of specific interest for the EURL activities and for DG SANCO: WG 3 “Method Validation” (2 meetings).

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 »