



Maisons-Alfort laboratory for food safety

2015 Work Programme of the European Union Reference Laboratory for Coagulase Positive Staphylococci

Version 1 – 13 October 2014

INTRODUCTION

In May 2006, the Laboratory for Food Safety, Maisons-Alfort location, 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 2015, according to the actions planned at the 8th Workshop of the National Reference Laboratories (NRLs) (4-6 June 2014).

Scientific & technical activities of EURL CPS are mainly undertaken, in the laboratory, by the Staphylococci Team of Unit Staphylococci, *Bacillus*, Clostridia & Milk (SBCL).

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 (SEs), requiring absence in 25 g in cheeses, milk/whey powder, to be tested when CPS enumeration is higher than 10⁵ 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 2015), 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: NRL training and support to the NRLs,
- Section 4: Technical and scientific assistance to the European Commission.

0. GENERAL ASPECTS

0.1 GENERAL COORDINATION (MUTI-ANNUAL)

General coordination by EURL management team, administrative department (SAG) of the NRL network: 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.

Missions:

1 mission at DG SANCO (Brussels, 1 day).

0.2 WORKSHOP OF THE NRLS (ANNUAL)

EURL CPS will organise the 9th Workshop of the NRLs in 2015, dedicated to staphylococcal enterotoxins.

This workshop will be organized at EURL CPS, Maisons-Alfort. Three experts would be invited, as well as NRLs from accessing countries.

DISPATCH OF METHODS AND PROFICIENCY TESTING TRIALS

1.1 STAPHYLOCOCCI DETECTION/ENUMERATION IN FOOD

1.1.1 PRODUCTION OF "NATURALLY" CONTAMINATED CHEESES FOR PT TRIALS (MULTI-ANNUAL)

Duration: 2015 – expected end: 2018

Objective

EURL CPS is studying food matrices to be used to prepare samples for proficiency testing (PT) trials on CPS enumeration for the NRL network. The ability of NRL to implement the Standard reference methods for CPS enumeration (EN ISO 6888-1, 2) may vary depending on the type of food matrices analysed and the corresponding sample preparation step. Up to now, EURL has used several types of food samples: either artificially contaminated samples (i.e. pasteurized milk, cheese, milk powder, shelled prawns) or naturally contaminated samples that were stored frozen (i.e. uncooked soft cheeses and pressed cheeses). However, the ability to enumerate CPS on selective agars can be different (in general better) when artificially contaminated samples are analysed, compared to naturally contaminated foods which are received and analysed by NRLs in routine.

Therefore, EURL CPS will investigate the interest of the pilot production, in an experimental plant, of "naturally" contaminated food samples to be used for PT trials. For this investigation, EURL CPS would select cheeses as food samples to be studied. "Naturally" contaminated cheeses could be produced in an pilot dairy plant using milk formerly inoculated with staphylococcal strains. The aim is to study distribution of CPS (see below) and of staphylococcal enterotoxins (SEs) (see section 1.2.2.) in the cheeses and to assess the suitability of these cheeses as material for PT trials. A collaboration with a laboratory having a pilot dairy plant is required.

Expected output and time of delivery

In 2015, EURL CPS will initiate a collaboration with the Italian NRL (IZSTO) to obtain "naturally" contaminated cheeses which would be produced in the laboratory dairy plant at the Italian NRL.

Over the time period 2015-2016, this study would be dedicated to the production of the contaminated cheeses, with a focus on the selection of the most suited CPS strains and of the optimal parameters allowing CPS development and SE production within the cheeses. Different types of cheeses would be produced and tested, such as soft fresh cheeses (weight of 1-5 kg) and ripened traditional cylindrical flattened cheese (weight of 8-12 kg). This study would include the analysis of CPS/SE distribution in the cheeses. Different portions of the cheeses would be tested (core versus periphery). Homogeneity and stability for CPS and SE would be tested.

Sub-contracting

The shipment of samples between Italian NRL and EURL will be sub-contracted.

Mission

1 mission at IZSTO (Torino, IT, 2 days).

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

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

Duration: 2011 - 2015

Objective

As solid food matrices are part of foodstuffs included in the microbiological criteria for CPS, in EC Regulation 2073/2005, it is thus necessary to organize PT trials on solid food matrices. EURL CPS has already organized PT trials on such sample types, but the artificial contamination technique should be optimized, as (i) to be easily implemented by the EURL and the NRLs, (ii) to be repeatable, as well as (iii) to ensure a satisfactory homogeneity and stability of CPS contamination.

In addition, according to the results obtained from the beginning of this study, it was decided to include a sub-sampling step of the test portions, to be performed by the participant laboratories, during the 2014 PT trial organized by EURL on CPS enumeration. Indeed, it is important to include this initial step of the analysis in PT trials, as it can have a major impact on the validity of the analyses of solid matrices.

Expected output and time of delivery

In 2013, EURL CPS, in collaboration with EURL *Listeria monocytogenes*, conducted a bibliographic review and an enquiry which allowed to collect experiences from the NRLs as PT trial organisers at national level.

In 2014, EURL CPS continued the experimental study launched in 2013, in which different inoculation techniques of solid food matrices were compared, in order to optimize the combination between the solid food matrix and the inoculation technique. In particular, EURL performed a contamination of shelled prawns in a batch system, combined with manual homogenization, which was subsequently studied in terms of homogeneity and stability.

As satisfactory results were obtained, the outcome of this study will be used by EURL CPS for future PT trials and it can also help the NRLs for the organization of their own interlaboratory PT trials at a national level.

Since this study has been conducted until now on prawns, and as it is necessary to assess whether the contamination technique selected is applicable to other types of solid food matrices, such as cheeses, this project will be extended in 2015. Promising additional contamination techniques, such as airbrush-based contamination, will also be tested in this study, with diced cheeses or sliced cheeses as experimental samples.

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 EC Regulation 2073/2005for CPS enumeration in food.

Expected output and time of delivery

In 2015, EURL CPS will organize an inter-laboratory PT trial on CPS enumeration by one or both reference methods EN ISO 6888-1 & 2, using powdered infant formulae as sample material.

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 PT TRIAL

Objective

The inter-laboratory PT trials organised by 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 2073/2005 for SE detection in food.

Expected output and time of delivery

EURL CPS will organize a PT trial in 2015 on SE detection in a food matrix (milk product) reported to be involved in staphylococcal food-borne outbreaks in Europe, using the applicable ESM version.

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

1.2.2 PRODUCTION OF "NATURALLY" CONTAMINATED CHEESES (MULTI-ANNUAL)

The study presented in 1.1.1 will be also undertaken for SEs, in order to investigate the interest of the use of "naturally" contaminated cheeses for PT Trials dedicated to SE detection.

Mission:

1 mission at IZSTO (Torino, IT, 2 days).

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

1.3 STAPHYLOCOCCI STRAIN CHARACTERIZATION AND TYPING

1.3.1 DISPATCH OF STRAINS (MULTI-ANNUAL)

Upon request of the NRLs and in order to implement methods for detection of *se* genes and for sub-typing of CPS strains, EURL CPS (Unit SBCL) would send CPS field strains and/or DNA material from its collection to the NRLs.

Sub-contracting

Transportation of the strains and/or DNA to the NRLs will be sub-contracted.

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

ANALYTICAL DEVELOPMENT

2.1 STAPHYLOCOCCI DETECTION/ENUMERATION IN FOOD

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

Duration: 2010 –2015 for part 2.1.1.1 and 2016 for part 2.1.1.2

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 as possible.

In the series of Standards EN ISO 6887-2 to 5 on the preparation of test samples for microbiological analyses of different types of food matrices, it is not specified how to sub-sample the test portion within the "laboratory sample" (i.e. sample that is sent to the laboratory). 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 of the test portion within solid food 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.

This study currently comprises two parts:

- Impact of test portion size on MU (2.1.1.1);
- Study of the distribution of CPS and SEs on MU (2.1.1.2).

The outcome of this study would be transferred to ISO/TC 34/SC 9, in charge of standardization in microbiology of the food chain:

- To provide data for the revision of ISO/TS 19036 (MU estimation for quantitative determinations), and to quantify MU part linked to sub-sampling of test portions for solid matrices,
- To revise EN ISO 6887 series to better define the procedure of test portion subsampling in solid matrices.

2.1.1.1 IMPACT OF THE TEST PORTION SIZE ON MEASUREMENT UNCERTAINTY

Expected output and time of delivery

EURL CPS began in 2014 an experimental study on sub-sampling cheeses' test portions of a size larger than 10 g (i.e. 100g), which included several protocols for the dilution steps. This study was performed in order to obtain a satisfactory MU associated with the chosen sub-sampling technique. The outcome of this study was transferred to ISO/TC 34/SC 9/WG 8, in charge of the revision of EN ISO 6887 series, to include more detailed guidance on test portion size in EN ISO 6887-1. The outcome was also discussed at the 2014 workshop: it was agreed that a 100 g test portion size would be difficult to implement in routine for official controls and that a 25 g size would be realistic.

At the 2014 workshop, it was also agreed that EURL would coordinate in 2015, in conjunction with EURL *Lm*, a collaborative study on the impact of test portion size (25 g versus 10 g) on CPS enumeration and MU. This study will require the participation of volunteering NRLs and national official laboratories. Analysis of some "naturally" contaminated cheeses could also be performed at EURL (see 1.1.1).

Mission

1 mission at IZSTO (Torino, IT, 2 days).

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

2.1.1.2 STUDY OF THE DISTRIBUTION OF STAPHYLOCOCCI AND STAPHYLOCOCCAL ENTEROTOXINS AND IMPACT ON MEASUREMENT UNCERTAINTY

Expected output and time of delivery

EURL CPS will complete over 2015-2016 the investigation study launched in 2011 on the heterogeneity of CPS and SE contamination in naturally contaminated cheese samples (i.e. samples obtained from own-checks analysis and stored frozen at EURL).

In 2015, EURL CPS will gather all the data obtained from the analyses (intra-batch variability) performed in 2011-2012 on soft uncooked cheeses ("Bleu de Gex") and on two different pressed uncooked cheeses ("Tome au marc" and "Morbier"). A statistical analysis of the results will then be performed in order to investigate CPS and SE heterogeneity in the samples and to evaluate its impact on MU.

At the end of 2015 and in 2016, EURL CPS will also complete the experimental study on the inter-batch variability initiated in "Bleu de Gex", by including the two other types of cheeses "Tome au marc" and "Morbier".

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 USE OF MASS SPECTROMETRY FOR CHARACTERISATION AND QUANTIFICATION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

Duration: (restart) <u>2015</u>-2018

Objective

The current EURL CPS confirmatory method is ELISA-based. The situation where both screening and confirmatory methods are based on the same principle is not satisfactory, all the more immuno-enzymatic, introduces a risk of bias in the confirmation of screening results. In order to avoid this risk, EURL CPS has previously conducted a collaborative project with CEA (French Alternative Energies and Atomic Energy Commission) to investigate an alternative tool to immunology, that is quantitative mass spectrometry (MS), in order to confirm and quantify SEs presence in food matrices.

Between 2011 and 2012, the Protein Standards Absolute Quantification (PSAQs) for the major SEs (types SEA, SEB, SEC, SED, SEE and SEG) were correctly transferred to EURL CPS. However, the development of the multiplex quantitative MS methodology using these PSAQs was not fully achieved due to the withdrawal of this project from EURL work program in 2013.

Currently an MS method for SEs is being developed in two NRLs, from Belgium and Sweden. The objective of the study is to re-start the development of a MS-based method for SE confirmation and quantification, in collaboration with these two NRLs.

Expected output and time of delivery

In 2015, EURL intends:

- To conduct a bibliographical study;
- To re-activate the MS method at the laboratory;
- To launch exchanges with SE and BE NRLs on the MS method developed in each laboratory;
- To test different reagents developed in each laboratory.

Missions

2 missions at SE-NRL (SVL, Uppsala) and BE-NRL (ISP, Brussels), 2 days each.

Subcontracting

Transportation of samples between EURL and NRLs will be subcontracted.

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

2.3 CHARACTERIZATION AND TYPING OF STAPHYLOCOCCI STRAINS, EPIDEMIOSURVEILLANCE

2.3.1 VALIDATION OF A MULTIPLEX PCR METHOD FOR SE GENES DETECTION (MULTI-ANNUAL)

Duration: 2011 – 2015

Objective

For the investigation of staphylococcal food-borne outbreaks (SFPOs), it is often necessary to detect the presence of *se* genes in CPS strains, in addition to SE detection in the suspected food, in order to confirm the identity of the food responsible for SFPO.

EURL CPS has been developing a multiplex real-time (RTi) PCR scheme for the detection of 13 se genes.

Expected output and time of delivery

In 2013, the EURL has evaluated the RTi-PCR scheme previously developed against a limited strain panel (63 strains), this work was carried out in collaboration with the UK-NRL (PHE). This evaluation was performed in comparison to EURL conventional PCR method. This study pointed out several discrepancies of RTi-PCR method, in comparison to the conventional PCR method, which required optimization of the RTi-PCR scheme in 2014, particularly by sequencing *se* genes and PCR products involved in the discrepant results. The specificity study has also been extended to a larger strain panel.

Based on these results, EURL CPS will conduct in 2015 a validation of the optimized RTi-PCR scheme. Seven NRLs have volunteered to collaborate with EURL for this study.

Subcontracting

Sequencing of discordant genes and transportation of DNA and/or strains to participating NRLs.

Mission

2 visits (2 days each) to UK-NRL (PHE, Colindale) for organization of the validation study.

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

2.3.2 MOLECULAR ALTERNATIVE METHODS TO PFGE (MULTI-ANNUAL)

Duration: (restart) 2015–2017

Objective

Previously, EURL CPS worked on the development of an alternative method to PFGE for the characterization of CPS strains. This was a Multi Locus Variable number tandem repeat (VNTR) Analysis (MLVA), coupled with capillary electrophoresis (CE). 16 VNTR loci have been selected, including those described by Pourcel *et al* (Journal of Clinical Microbiology, 2009, 3121-3128). This protocol was tested on a large panel of strains including reference and field food strains. The results obtained showed that MLVA was as discriminatory as PFGE. This project was deleted from EURL CPS work programme in 2013.

MLVA thus represents a promising method which would be suitable for characterization of CPS involved in SFPOs.

Expected output and time of delivery

For a few VNTR, EURL observed some difficulties for MLVA data interpretation, and EURL will optimize the method.

In addition, the MLVA protocol requires the use of a kit commercialized by CEERAM (France), which includes two costly sets of labelled oligonucleotide primers. EURL CPS will therefore develop an alternative "in-house" kit and to improve the MLVA protocol, in order to develop a EURL CPS method that it would share within the NRLs network for SFPO characterization.

Subcontracting

The implementation of MLVA technique will require subcontracting for gene sequencing of CPS strains.

Mission

3 missions, 1 day each (CEERAM, Nantes, FR).

Capital equipment

Gel analyser to be acquired.

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

NRL TRAINING AND SUPPORT TO THE NRLS

3.1 DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOOD

3.1.1 EUROPEAN SCREENING METHOD

Duration: 2015

Objective, expected output and time of delivery

In 2015, the EURL CPS intends to organize for NRLs one (or several) training session(s) on SE detection, according to the revised ESM method (Method Anses Maisons-Alfort CAT BAC 06), depending on NRL needs, and in particular in case of unsatisfactory results obtained by certain NRLs during the 2014 PT trial (see 1.2.1).

Training

Travel and stay expenses of at maximum 3 trainees.

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

3.1.2 QUANTITATIVE ELISA METHOD

3.1.2.1 SUPPORT TO THE NRLS (MULTI-ANNUAL)

Objective: see 2.2.1.

Expected output and time of delivery

Upon request and for the NRLs which have not yet been trained to the EURL CPS ELISA method for SE quantification (method Anses Maisons-Alfort CAT-BAC 16), EURL will provide scientific and technical assistance to the NRLs, especially to perform confirmation analysis of positive screening results obtained by NRLs or official laboratories with ESM, in the frame of (i) official controls performed according to the SE criterion of EC Regulation 2073/2005 modified, or (ii) in case of SFPOs, upon request of the concerned NRLs.

Mission

1 mission is scheduled for a NRL which would need on-site assistance for implementation of the ELISA quantitative method.

3.1.2.2 NRL TRAINING

Duration: 2015

Objective, expected output and time of delivery

In 2015, EURL CPS intends to organise for NRLs two training sessions on SE quantification, according to the EURL CPS quantitative ELISA method, depending on NRL needs.

In particular, training of NRLs would enable to qualify them to participate to the inter-lab study for the characterization of certified reference materials (see 3.1.3).

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

3.1.3 DEVELOPMENT OF CERTIFIED REFERENCE MATERIALS: CERTIFICATION STEP

Duration: 2011-2015

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 EC/JRC/IRMM, dated 21/04/2010.

JRC/IRMM (Geel, BE), in collaboration with EURL CPS, has started in 2011 the project to develop CRMs on SEs in a lyophilized cheese matrix.

In 2013 and 2014, EURL performed analyses of three different CRM batches (blank, level 1 and level 2) dedicated to the homogeneity, short and long term stability studies. The results obtained showed that the material is homogenous and stable, meaning that the certification study could be carried out.

Expected output and time of delivery

The certification study will be conducted in two steps:

- i) Analyses using ESM v5: about 10 laboratories will participate to this step, using one of the two validated kits, Vidas SET2 and Ridascreen SET Total. Results are expected by the end of September 2014.
- ii) Analyses using the EURL CPS quantitative ELISA method (method Anses Maisons-Alfort CAT-BAC 16). The objective of this step is to assign a value to each level of contamination of the future CRMs. 10 NRLs should participate to this step. However, only four NRLs have been trained on the EURL CPS quantitative ELISA method.

In 2015:

- EURL will train 6 other NRLs on the EURL CPS quantitative ELISA method, at the beginning of 2015. Two sessions will be necessary. See 3.1.2.2.
- Before starting the certification study and in order to check the ability of these NRLs
 to implement the quantitative ELISA method in their laboratory, EURL will organize a
 a performance checking inter-lab trial for the 10 participants in the certification
 study.

Sub-contracting

Dispatch of samples to NRLS for performance checking inter-lab trial.

3.2 CHARACTERIZATION OF STAPHYLOCOCCI STRAINS

3.2.1 TECHNICAL ASSISTANCE TO NRL (MULTI-ANNUAL)

Objective, expected output and time of delivery

EURL CPS will ensure scientific and technical assistance on characterization of CPS strains (detection of *se* genes and typing of CPS strains), for NRLs upon request.

Subcontracting

Transportation of strains and sequencing of DNA, upon request.

Mission

1 mission (2 days) for a NRL which would need on-site assistance for implementation of the methods for CPS characterization.

3.2.2 TRAINING

Duration: 2015

Objective, expected output and time of delivery

In 2015, EURL CPS will organize a training session for the NRLs dedicated either (i) to the detection of *se* genes in CPS strains by conventional PCR, (ii) to typing of CPS strains by PFGE or (iii) to *spa*-typing of CPS strains, according to the need to be specified by the NRLs.

Subcontracting

Sequencing of DNA for training material.

Capital equipment

Gel analyser to be acquired.

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, participation of the EURL CPS, for the analytical aspects, to the update of EC Regulation 2073/2005 on microbiological criteria related to CPS and SEs, and any new question which may arise during the year.

Mission: 1 meeting in Brussels (1 day).

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 9¹ & CEN/TC 275/WG 6² 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" and WG 3 "Method Validation"
 - **Missions**: 1 meeting of WG 2 (2 days in Europe, TBD) and 2 meetings of WG 3 (2 days each in Europe, TBD).
- Participation of a EURL CPS scientist (Alexandra CAUQUIL) to WG 13 of ISO/TC 34/SC 9, in charge of (i) preparing an amendment to EN ISO 6888-1 (CPS enumeration, Baird Parker agar), to include an optional confirmatory test by stab-inoculation of RPF agar, and (ii) revising EN ISO 6888-3 (CPS detection/quantification by MPN) (no meeting outside Paris area).
- Leadership by a EURL CPS scientist (Jacques-Antoine HENNEKINNE) of the TAG 12 on SE detection of CEN/TC 275/WG 6, also 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

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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 »