EURL – Campylobacter

Work programme for 1st of January 2014 to 31st of December 2014

INTRODUCTION

The activities in the work programme for 2014 for the EU Reference Laboratory (EURL) - *Campylobacter* will follow EU legislation Regulation (EC) No 882/2004 and Commission Regulation (EC) 776/2006. The work programme includes description of activities, objectives, expected outputs and performance indicators. The performance indicators are included in a separate annex (Annex Performance Indicators).

The work programme for 2014 will consist of the following key activities:

1. Organisation of proficiency tests
2. Production and validation of analytical methods
3. Training and support to NRLs
4. Provision of expertise to stakeholders (EU Commission and agencies, Member States, candidate and third countries) and preparedness of staff for emergency situations
5. Reciprocal exchange of information with professional bodies
6. Development activities in the field of molecular methods for species identification and typing/strain characterization of *Campylobacter*
7. Communication

ACTIVITY 1
ORGANISATION OF PROFICIENCY TESTS, PTS, IN 2014

Regulation (EC) No 882/2004, Article 32 1b, 4a, b, d

Objectives: To provide NRLs with details of relevant analytical methods for performing PTs that mimic realistic diagnostic samples to be analysed for *Campylobacter* in the MSs. To assess the performance of the NRLs and to identify potential analytical problems that could be solved by assistance from the EURL in order to improve the performance.

The EURL has so far organised 12 proficiency tests for the NRLs. In addition to the NRLs in the EU MSs, three to four Official Laboratories (OLs) in third countries have participated in the PTs each year. The PTs have been developed to correspond to the type of analyses that are common in official control of *Campylobacter* in the food chain in the EU Member States.

Six PTs (including PT 11 in 2013) have included both detection and enumeration of *Campylobacter* in chicken skin, chicken meat or minced meat. Basically, the protocols for analysis (the SOPs) have followed the standardised protocols of ISO 10272 Part 1 and Part 2: 2006 "Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp".
In 2013, a new type of PT with sock samples was introduced. PT 12 consisted of sock samples with chicken faeces inoculated with live *Campylobacter* in culture broth (or blanks with un-inoculated culture medium). Using boot socks for collecting environmental samples from broiler houses has lately become common practice in many countries and it was therefore considered relevant to prepare a PT simulating this type of sample.

In 2014, the EURL plans to organise two PTs that will be similar to PT 11 and PT 12, in order to make it possible to compare NRLs´performances between the different years. Both PTs are considered to be of complexity grade 3. Both PTs will be distributed together, by courier in the spring 2014. Details about the PTs and the exact date for distribution will be discussed with the NRLs at the workshop in September 2013.

NRLs with poor performance will be contacted and the EURL will provide assistance to solve the problems leading to the poor performance. If there has been a delay in distribution or the package with the PT has been damaged, a new PT will be sent out. If the NRL asks for more 'hands-on help', the EURL staff will suggest making a mission to the NRL or NRL staff will be offered to visit the EURL for training.

PT 13, "Detection and enumeration of Campylobacter in food"

The planned proficiency test number 13 will consist of detection and enumeration of *Campylobacter* in a food matrix for example chicken meat, carcass skin or other relevant matrix, basically using the abovementioned ISO 10272 standards. Vials with freeze dried bacterial cultures will be used as reference material. The matrix will be thoroughly tested for stability and to ensure freedom from *Campylobacter* before the test is distributed to the NRLs.

PT 14. "Detection and species identification of Campylobacter in environmental samples"

The planned proficiency test number 14 will consist of detection and species identification of *Campylobacter* in environmental samples, such as simulated 'sock samples' containing for example chicken faeces, and/or other environmental samples collected by eg. swabbing a surface. The samples will represent the types of samples that are taken in and around chicken houses or slaughter houses for detection of *Campylobacter*. The matrices (chicken faeces or environmental material) will be collected from a *Campylobacter* free chicken flock and/or surfaces and thoroughly tested to ensure freedom from contaminating *Campylobacter*. The socks/swabs will be inoculated with broth culture containing live *Campylobacter*. The concentration and species of *Campylobacter* (bacterial reference material) will be determined in Activity 2.1. The method for detection will basically follow the ISO 10272 part 1: 2006 standard. The NRLs will be free to use any method (phenotypic or molecular) for identification of *Campylobacter* species.

The EURL will prepare standard operating procedures (SOPs) for the two PTs. The reporting of test results will be made by using an online service, QuestBack, which has been used for this purpose since 2012. Results will be analysed by relevant statistical methods. Preliminary reports of the results will be prepared and sent to the NRLs shortly after the deadline for submitting the results. The results will be presented and discussed at the workshop in 2014. Final reports will then be prepared and communicated with the participating laboratories and DG-Sanco.

Expected output: Two PTs of complexity grade 3 will be organised in 2014. All EU NRLs are expected to participate in both PTs. OLs in BA, CH, IS, MK and NO will be invited to participate. It is expected that > 75% of the participating laboratories provide results that are graded as 'acceptable' or higher in both PTs.

ACTIVITY 2
PRODUCTION AND VALIDATION OF ANALYTICAL METHODS

Regulation (EC) No 882/2004, Article 32 1a, 1c, 4a, b, e

Objectives: To provide information about new or modified methods for analysis of *Campylobacter* in new type(s) of sample (matrix) and to validate and/or participate in validation studies of methods.

2.1. Validation of methods and reference materials for preparation of PT 14, "Detection and species identification of Campylobacter in environmental samples"

For PT 12 with sock samples in 2013, different procedures were tested with both the matrix (socks with chicken faeces) and bacterial strains in order to, if possible, establish "standard" sock samples for the PT. It was concluded that the vials with freeze-dried *Campylobacter* that have been used for other PTs were not suitable and therefore live bacteria were used instead. Several *Campylobacter* strains and species were tested but it was difficult to find suitable isolates that worked well with the PT. This

work will continue in 2014, in order to build up a collection of *Campylobacter* strains of different species that can be used for future sock and swab sample PTs. Other components will also be more tested such as the size of inoculums and stability of the test.

Expected output: A protocol and a draft ”standard operating procedure” for the sock sample test was prepared in 2013. The work with a protocol also including swab samples is planned for 2014 with the aim to have selected the most suitable bacterial strains (bacterial reference material), optimized the concentration of bacterial inoculums to socks/swabs, and ensured the stability of the test.

2.2. Participation in a validation study of ISO 10272

Validation studies of ISO 10272 Part 1 and Part 2: 2006, were organized by the Food and Consumer Product Safety Authority and National Institute for Public Health and the Environment, The Netherlands, in 2013. The EURL collaborated in the studies and will continue to contribute with expert advice in 2014 when the results of the studies are evaluated.

Expected output: Reports of the evaluations will be prepared by the organizers and the EURL will contribute to this activity.

2.3 Study of a real-time PCR assay for detecting Campylobacter in caecum samples

A comparative study has been initiated in 2013 of a *Campylobacter* real-time PCR validated for detection of thermotolerant *Campylobacter* in chicken raw meat, faeces on cloacae swabs and on disposal shoe covers with chicken faeces (NordVal Certificate no 017). In the comparative study, the realtime PCR is tested with caecum samples which are also cultured by routine methods on mCCDA for comparison. The study is expected to be finished and summarized in 2014.

Expected output: A summary of the results will be communicated with the NRLs and other laboratories that monitor caecum samples from chicken for the detection of *Campylobacter*.

2.4. Detection of Campylobacter in water samples

Contaminated water has been shown to be a vehicle for the transmission of *Campylobacter* to humans, both in sporadic cases and large outbreaks. Contaminated water could also be a way to introduce *Campylobacter* to broiler flocks. The ISO 17995: 2005 standard “Water quality – Detection and enumeration of thermotolerant *Campylobacter* species” specifies a method for the detection and semiquantitative enumeration of *Campylobacter* in filterable water samples. However, the isolation of *Campylobacter* from water poses several problems. Large volumes of water are often required to reach desired sensitivity. Modifications of the ISO standard or new approaches for testing have been tried by many laboratories. The EURL plans to compare different methods or protocols for water analysis in 2014.

Expected output: Summary of the results of testing and comparing different methods for detection of *Campylobacter* in water will be communicated to the NRLs and other OLs.

ACTIVITY 3
TRAINING AND SUPPORT TO NRLS

Regulation (EC) No 882/2004, Article 32 1a, 1c, 1d, 4a –c, e, f

Objectives: To communicate, with NRLs, OLs and stakeholders, about ongoing activities that include *Campylobacter* at EU and national levels. To assist NRLs with scientific and technical advice and to train NRL staff in conventional and molecular techniques for *Campylobacter* analyses.

3.1 Organisation of a workshop

Description of planned workshop in Uppsala in 2014

The plan is to invite representatives from the 28 MSs NRLs for *Campylobacter* and up to ten persons from EU Candidate and third countries (Bosnia and Herzegovina, FYROM, Iceland, Norway, Switzerland, and Turkey) as reimbursed participants. As in previous years, experts from DG- SANCO, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) will be invited and asked to present *Campylobacter* activities at EU level.

The agenda will include presentations and discussions on:

- *Campylobacter* activities in the EU at Community level. Results of zoonosis monitoring, surveys and control of *Campylobacter* in animals, food stuffs and humans
- Results of proficiency tests
- Updates on analytical methods, including validation/assessment of methods for detection and enumeration of *Campylobacter* and molecular methods for identification and characterization of *Campylobacter* strains
- *Campylobacter* activities at national level (EU MSs and third countries), i.e. monitoring and research studies
- Information about proficiency tests to come
- Information from meetings and activities within working groups of ISO/CEN, ISO/TC34/SC9 and CEN/TC 275/WG6
- Information about the revised ISO 10272 standards
- Future EURL-*Campylobacter*- NRL collaboration and activities, e.g. training activities, depending on recent and urgent matters of common interest

At least one NRL representative from each EU MS is expected to participate in the workshop in 2014. Actions taken to ensure participation include:

- A date for the workshop in 2014 will be suggested already at the workshop in 2013 (First announcement)
- A second announcement with details will be sent out about 4 months before the workshop
- Reminders will be sent out by emails and if necessary be made by phone
- If an NRL is unable to participate, The EURL will contact them and ask the NRL to provide a written explanation for the reasons why not attending the workshop.

From third countries' OLs, five to six experts are expected to participate.

In previous evaluations of workshops, the majority of participants have given high points and positive comments about the workshops. Actions to address negative feedback will include discussion within the EURL to evaluate the feedback and possibilities to make necessary changes. The EURL may contact the NRLs or make a survey by use of QuestBack to find ways to change things that have received low points or negative comments in the evaluation of the workshop.

Expected output: Representatives from all EU MSs NRLs- Campylobacter and from OLs in 5 to 6 third countries will participate and positive responses will be given in the evaluation survey by the majority of participants. Presentations given at the workshop and a summary of the workshop will be posted on the website.

3.2 EURL staff visits (missions) to NRLs for training of NRL staff
If an NRL repeatedly underperforms with the *Campylobacter* analyses in the PTs, the EURL will suggest a visit to the NRL for training of the staff. Before the mission, the EURL staff will prepare laboratory material, relevant literature and presentation material needed for the visit.

Expected output: Depending on the situation, one such visit (mission) is planned for 2014.

3.3. Training course and study visits to EURL
A training course in the application of molecular techniques is planned to be organized for a maximum of 5 participants in 2014. The training course will either be focused on PCR for identification of thermophilic *Campylobacter* spp or PFGE technique for the strain characterization (typing) of *C. jejuni*.

If requested and on ad hoc basis, the EURL will offer training for NRLs that plan to make study visits to the EURL.

Before a training course or an ad hoc training activity takes place, preparations will be made by the EURL, i.e. testing assays, bacterial strains, making up laboratory protocols and lists of suppliers of reagents, chemicals, equipment, etc., and collect relevant literature for the participants/visitors.

Expected output: One training course or ad hoc training activity is planned for 2014

3.4. Ad hoc assistance to NRLs
Upon request from the NRLs, the EURL will perform confirmatory testing of isolates that the NRLs send to the EURL. Usually, the NRL asks for species identification and the number of submitted isolates per year has ranged from 1 to 30 from a single laboratory. The EURL also provides assistance on questions about methodology, techniques, equipment, etc. NRLs are also provided with "reference material" consisting of well characterized strains from the EURL, to help when the NRL is setting up a new method, for example PCR or typing by a molecular method.

Expected output: It is difficult to foresee how many requests will be made, but the EURL always provides assistance as soon as possible when these types of questions occur.

3.5. Preparation of learning material for the website
For some NRLs, changing of staff and/or limited experience of routine analysis of *Campylobacter* could be reasons for poor performance of PTs. Some steps in the standard analysis of *Campylobacter* are more problematic than others. Phenotypic tests for confirmation and species identification are sometimes misinterpreted and some NRLs have problems with enumeration of *Campylobacter* on agar plates with contaminating flora. The EURL will prepare photos and text material to be presented at the website, starting with basic steps in the analysis following the standard ISO 10272 Part 1 and 2 (2006). The website material will be a useful complement to other assisting activities provided by the EURL, such as training courses and missions to NRLs. The preparation of learning material for the website will start in 2014 and be continued in coming years where necessary.

Expected output: Texts and photos demonstrating details in the basic procedures of *Campylobacter* analysis according to the ISO 10272 procedures will be prepared and posted on the website.

ACTIVITY 4
PROVISION OF EXPERTISE TO STAKEHOLDERS (COMMISSION AND AGENCIES, MEMBER STATES, CANDIDATE AND THIRD COUNTRIES) AND PREPAREDNESS OF STAFF FOR EMERGENCY SITUATIONS

Regulation (EC) No 882/2004, Article 32 1e, 1f, 4a, 4e, 4h

Objectives: To ensure that the EURL staff is well trained, up-dated and knowledgeable about the area of *Campylobacter* so that appropriate expertise can be provided to stakeholders and emergency situations can be handled in a proper way.

4.1. Provision of expertise to stakeholders
Requests from the Commission and agencies for scientific and technical assistance will have priority and be handled by the EURL scientific staff in a timely manner.

One person of the EURL staff (Elina Lahti) will continue to be a member of the EFSA Task Force on Zoonoses Data Collection in 2014.

On request, EURL staff will act as tutors at training programmes and lecturers at seminars, for example at Microbiology courses for third countries within the European Training Platform for Safer Food Programme (DG SANCO), and workshops organised by TAIEX.

Campylobacteriosis is one of the diseases in focus for ECDC's Programme on Food and Waterborne Diseases and Zoonoses (FWD). The EURL will continue to collaborate with ECDC and provide assistance in the work with harmonizing surveillance including analytical methods for campylobacteriosis in humans.

Meetings with the Commission services that are of relevance for EURL staff to participate in:
- One coordination meeting of EURLs in the area of veterinary public health- biological risks, organized by DG- Sanco.
- One meeting with Commission working groups under the Standing Committee on the Food Chain and Animal Health (SCFCAH), section biological safety of the food chain in Brussels.

Expected output: Scientific and technical support will be given to stakeholders

4.2. Preparedness of staff
To ensure high quality and competence within the area of *Campylobacter*, the issues of skills of the EURL staff and continuous professional development are of fundamental importance. The EURL staff will thus collaborate with and visit other expert laboratories and participate in international and national networks, scientific seminars, conferences and workshops, ie:
- One member of the EURL staff (Eva Olsson Engvall) is a member of the Advisory Board to the EU FP7 financed project "*Campylobacter* control – novel approaches in primary poultry production" (acronym: CamCon, http://www.camcon-eu.net/). The four-year project started in 2010 and was recently approved an extension with one year. Project coordinator is Merete Hofshagen, National Veterinary Institute, Norway.
- Relevant national and international seminars and research meetings in order to assure competence and knowledge on recent advancement within the *Campylobacter* area.
  In 2014, members from the EURL staff plan to participate in the international conference
  Food Micro in Nantes, France 1-4 September (http://www.foodmicro2014.org/).
  This conference is organized every second year, with the scientific support of The International Committee on Food Microbiology and Hygiene (ICFMH).
- Med-Vet-Net Association (MVNA). The European Network of Excellence Med-Vet-Net ended in 2009 and an Association with the same name was formed. The EURL will participate in relevant activities of MVNA. If possible, one EURL staff member will attend the next Med-Vet-Net conference which will be held in collaboration with the Society for Applied Microbiology (SFAM) from 30th June to 3rd July 2014 in Brighton, UK.

Expected output: The members of EURL staff will maintain high competence and acquire new important knowledge in the field of *Campylobacter*.

ACTIVITY 5

RECIPROCAL EXCHANGE OF INFORMATION WITH PROFESSIONAL BODIES

Regulation (EC) No 882/2004, Article 32 1f, 4e

Objectives: To exchange information and assist with expertise when requested from professional bodies, and to actively participate in CEN/ISO standardization activities

Provision of consultant expertise to FAO/WHO/OIE
The EURL- *Campylobacter* is not a reference laboratory for FAO/ WHO, or reference laboratory or collaborating centre of OIE, but provides consultant expertise on an ad hoc basis to these professional bodies whenever requested.

Participation in CEN/ISO activities
EURL staff participates in CEN/ISO standardization activities and one staff member (Ingrid Hansson) is active member of working groups:
- Working group CEN/TC 275/WG 6/TAG 5
- Revision of ISO 10272: 2006, Part 1 and Part 2

The following meetings will be attended in 2014:
- The 33rd meeting of ISO/TC34/SC9 and the 21st meeting of CEN/TC275/WG6, which will be held in Washington DC, USA. Total duration of the two meetings will be 5 days.
- One meeting with working group CEN/TC275/WG6 TAG 5 "ISO 10272 standards", date not set yet. Duration is probably 2 days.

Expected output: Reports from the meetings will be prepared and the EURL will contribute to this activity.

ACTIVITY 6
DEVELOPMENT ACTIVITIES IN THE FIELD OF MOLECULAR METHODS FOR SPECIES IDENTIFICATION AND TYPING/STRAIN CHARACTERIZATION OF *CAMPYLOBACTER*

Regulation (EC) No 882/2004, Article 32 1a, c, 4a, g, h

Objectives: To achieve more experience and knowledge about molecular methods for detection, identification and strain characterization ("typing") of *Campylobacter* in order to provide the NRLs with details about the methods and advances in the field.
Further, to prepare routines for handling molecular typing data from NRLs.

6.1. Methods for species identification
Species identification of *Campylobacter* by traditional phenotypic tests ("biochemistry") is usually not as reliable as molecular methods. This has been very obvious in all PTs that the EURL has organized. When the EURL receives strains from the NRLs for confirmation and species identification, a set of tests, mainly PCR- based assays, are used in order to obtain a conclusive identification. The EURL will continue to evaluate PCR assays and other non-cultural methods, ie mass spectrometry (MALDI-TOF) for species identification of *Campylobacter*.

6.2. Methods for strain characterization of Campylobacter
Strain characterization or 'typing' of *Campylobacter* isolates is important, especially when studying outbreaks of food borne infections and for the identification of transmission routes for example from an animal source. Although campylobacteriosis cases are often considered to be sporadic events, larger food-borne outbreaks have also recently been identified, much thanks to the use of molecular typing methods.

A 'Vision paper on the development of data bases for molecular testing of fooborne pathogens in view of outbreak preparedness' was prepared by DG-Sanco in 2012. In this document, it is stated that molecular typing of food-borne pathogens could "substantially contribute to the epidemiological investigations of foodborne outbreaks and to the identification of emerging health threats". An initiative to collect molecular typing data (PFGE) from food-, animal-, and human isolates in two data bases was presented with EFSA managing food and animal and ECDC managing the human typing data. In the pilot project, only four pathogens are included (not *Campylobacter*), but it is expected that also *Campylobacter* will be in focus for this activity, since campylobacteriosis is one of prioritized diseases by ECDC.

Many NRLs- *Campylobacter* perform molecular typing but there is a need for harmonization of methods and reference materials. The EURL- *Campylobacter* often receives questions about what protocols, equipment and other material should be used.

To be prepared for the expected extension of the EFSA-ECDC databases to cover *Campylobacter* isolates and to be able to provide technical assistance to the NRLs it is important that the EURL is updated on the techniques and has experience and knowledge about details of the methods. At the EURL, three techniques for molecular typing are being used and tested to gain experience and good knowledge of each type of technique.

Pulsed field gel electrophoresis, PFGE

Two standardised protocols are recommended for use:
Campynet protocol (http://campynet.vetinst.dk/PFGE.html) which has been most often used by the EURL.
PulseNet (USA- PulseNet) protocol (http://www.cdc.gov/pulsenet/PDF/campylobacter-pfge-protocol-508c.pdf) which, at international level is more commonly used. At the EURL- *Campylobacter* PFGE training course in 2011, this was the protocol that was trained.

The EURL has compared the two protocols and found that they both perform well, giving comparable results. However, if PFGE data will be collected at EU level, the PulseNet protocol will be recommended and supported by the EURL.

Multi locus sequence type, MLST

The MLST method according to reference Dingle et al (2001) (1) has been established at the EURL. The details of the protocol are available at http://pubmlst.org/campylobacter/ . The PubMLST website also holds the database for sequences, determining the designations of the ST types. The advantage with MLST is that sequence data are unambiguous and can be exchanged between laboratories and compared with the big database at the PubMLST website.

Whole genome sequencing, WGS

WGS has been an extremely expensive technique for characterization of bacterial strains. However, the costs are declining and the methods available for handling the large amount of sequence data have increased. At a small scale, the EURL has in collaboration with the NRL- *Campylobacter* at SVA, started to test the technique using MiSeq (Illumina) in order to meet future needs for assistance and advice from NRLs and stakeholders. At DTU, Denmark, a web-based method has been developed for extracting relevant information of whole genome sequences to be used for determination of "MLST types" (2). The EURL will explore the possibilities to gain more information from WGS data in 2014 by testing a selection of *Campylobacter* strains in collaboration with some NRLs.

The EURL collaborates with the Swedish NRL- *Campylobacter* in research projects that among other things include strain characterization by molecular techniques.
The EURL staff provides competence and expert advice on methodology and interpretation of results. In return, the EURL staff gains updates and valuable knowledge about relevant research questions. A repository of typing data is being developed, covering Swedish animal and environmental

Campylobacter isolates. This repository could easily be adjusted to include also typing data from NRLs.

Expected outputs: More knowledge will be acquired about analytical methods for detection and identification of *Campylobacter* and about strain characterization by molecular methods. Routines for handling incoming isolates for typing and typing data will be established.
Publications: members of the EURL staff will author/co-author 2-4 scientific publications in peer reviewed journals and contribute with oral/poster presentations at scientific meetings, eg. the Food Micro conference in Nantes in September 2014.

ACTIVITY 7
COMMUNICATION

Regulation (EC) No 882/2004, Article 32 1a- f, 4b-c, g

Objective: To communicate with the Commission and its agencies, with NRLs, OLs and stakeholders and provide quick assistance in a user-friendly way.

The website is used for communication of relevant information and will be improved in 2014. The EURL will maintain and continuously update the list of NRLs- *Campylobacter* contact persons in EU MSs and at the corresponding official laboratories in other European countries that are participating in activities organized by the EURL- *Campylobacter*. Presentations given at the workshop will be posted as pdf-files on the website and technical information about PTs will be provided.

Most communication with NRLs, the Commission, other EURLs and stakeholders is done by emails and consists of both short questions and more complicated issues, sometimes on ad hoc basis. The time right before and after workshops and PTs are the periods with most intensive contacts with NRLs. The web based form for reporting results of PTs (QuestBack) is very useful and has been well received by the NRLs. The reporting form is designed to fit each individual PT and the NRLs are encouraged to send their comments in order to make improvements. The EURL prepares draft and final reports of the PT results which are then distributed to the NRLs and DG- Sanco by email. The annual technical and financial reports are sent to DG-Sanco both by regular post and email.

Expected outputs: The website will be updated and improved. Technical and financial reports for 2013 will be sent to DG-Sanco by deadline (31 March 2014) and the final PT reports will be sent to the NRLs after the workshop in 2014.

References

1. Dingle KE, et al. 2001.Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiology,39: 14-23.
2. Larsen MV, et al 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J. Clin. Microbiol, 50: 1355.