EURL- *CAMPYLOBACTER*

WORK PROGRAMME FOR 1st OF JANUARY 2012 TO 31st OF DECEMBER 2012

Introduction

The activities in the work programme for 2012 for the EU Reference Laboratory (EURL) - *Campylobacter* will follow EU legislation on CRLs (now EURLs) functions, duties and designation (Regulation (EC) No 882/2004 and Commission Regulation (EC) 776/2006).

The work programme for 2012 will consist of the following key activities:

1. Proficiency test(s)
2. Workshop
3. Research and development
4. Scientific and technical assistance to the European Commission and to the NRLs-*Campylobacter*, including ad hoc activities
5. Missions
6. Communication
7. Meetings
8. Training activities

1. Organisation of proficiency test(s), PT(s), in 2012

Short background
The EURL has so far organised 8 proficiency tests for the NRLs. Four tests have included both detection and enumeration of *Campylobacter* in chicken skin, chicken meat and 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". The majority of NRLs have performed very well with the analyses. The tests 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 MSs.
The aims with organising PTs are 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.

1.1. Detection and enumeration of *Campylobacter* in meat matrix

The planned proficiency test in 2012 will consist of detection and enumeration of *Campylobacter* in meat matrix (chicken meat or minced meat), basically using the abovementioned ISO standards. The ISO 10272 standards are under revision and therefore other enrichment broths and incubation times and temperatures may be included in the proficiency test. Suitable modifications to be tested will be discussed at the EURL-*Campylobacter* workshop in October 2011. Vials with freeze dried bacterial cultures will be

used as reference material. The meat matrix will be thoroughly tested to ensure freedom from *Campylobacter* before the test is distributed to the NRLs.

The EURL will prepare a test protocol (SOP) and test report. The reporting of test results will also be possible by using an online service, QuestBack, which has been acquired in 2011. Results will be analysed by relevant statistical methods. A report of the summarized results will be prepared and sent to the NRLs and to DG- Sanco. The results will also be presented and discussed at the workshop in 2012.

The PT is planned to be distributed by courier in the spring 2012. The exact date for distribution will be discussed with the NRLs at the workshop in October 2011.

1.2 Detection and identification of *Campylobacter* in swab samples.
Possible Ad hoc proficiency test

An additional proficiency test may be organised with detection and identification of *Campylobacter* in swab samples. The need and extent of an additional proficiency test will be discussed at the workshop in October 2011.

2. Organisation of a workshop

A workshop will be organised in the fall (probably in October) in 2012. Representatives from the Member States´ NRLs for *Campylobacter* will be invited and up to ten persons from EU Candidate Countries and third countries (Croatia, Bosnia and Hercegovinia, Iceland, Norway, Serbia, Switzerland, and Turkey) will also be invited to participate 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. In addition, up to three experts will be invited as speakers on topics of immediate interest for the NRLs.
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 test(s)
- 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 in MSs, including national 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 revision of ISO standards
- Future EURL-*Campylobacter*- NRL collaboration and activities, e.g. training activities, depending on recent and urgent matters of common interest

The aims with organising the workshop are to inform the NRLs about ongoing activities that include *Campylobacter* at EU and national levels. Further, to present and discuss PT results and efforts to improve diagnostic skills. The experts from the NRLs will be encouraged to take active part in discussions and to share information and experience about own *Campylobacter* work.

3. Research and development

This section includes a variety of laboratory work, both for the development of PTs and for gaining more experience of analytical methods for the detection, identification and characterization of *Campylobacter* as well as other issues that are important for increasing the knowledge about *Campylobacter* in the food chain.

3.1. Studies on bacterial and matrix reference materials for the proficiency test(s).

3.1.1. Detection and enumeration of *Campylobacter* in meat matrices

As for the proficiency test in 2011, the EURL plans to purchase vials with freeze dried reference material consisting of *Campylobacter* and other bacterial species of different and specified concentrations. The EURL will test batches of the freeze dried reference material with selected matrices and using different cultural conditions (enrichment broths, incubation times and temperatures) to ascertain the stability of the test procedure. The PT will be delivered by courier, a procedure that normally means a maximum transport time of 48h. However it could take longer to some destinations and the EURL therefore has to prepare samples that are stable for at least 4 days.

3.1.2. Detection and identification of *Campylobacter* in swab samples. Possible Ad hoc proficiency test

For an ad hoc proficiency test, selected isolates will be thoroughly tested with phenotypic tests and PCR-based assays. The isolates will be tested for stability and sent as swab samples in Amies transport medium containing charcoal. The swabs will be transported by courier.

3.2. Validation study.

ISO 10272 Part 1 and Part 2:2006 have not been validated in collaborative tests. The proficiency test 2011 served as a pre-study for validation. The results will be discussed at the workshop in October 2011 and the need for further validation studies will be investigated and planned to be carried out in 2012.

3.3. DNA-based (molecular) methods for detection, species identification and strain characterization of *Campylobacter.*

Short background

The traditional methods for detection and identification of *Campylobacter* include culture in selective media, followed by phenotypic methods for genus and species identification. Although molecular or DNA based methods are considered more reliable, there are no ‘golden standard’ methods for detection, species identification or strain characterization (subtyping/fingerprinting).

PCR-based tests for detection of *Campylobacter* in food items have recently become commercially available. An increasing number of NRLs are now using PCR-based assays for identification of *Campylobacter*. The EURL is often asked by the NRLs for guidance which test they should choose and there are requests from the manufacturers to evaluate their tests too.

3.3.1. PCRs for detection and identification of *Campylobacter*.

The EURL will perform comparative studies and assess PCR-based methods in order to assist the NRLs. In collaboration with Bio-Rad, a realtime PCR (iQ-Check *Campylobacter* Kit) for detection of *C. jejuni*, *C. coli* and *C. lari* in food has been studied in 2011 and the work will

continue in 2012. Both traditional PCR and realtime PCR assays will be reviewed and selected for further testing, preferably in collaboration with some NRLs on a voluntary basis.

3.3.2. Subtyping/fingerprinting

For subtyping by Pulsed-field gel-electrophoresis (PFGE), the standardised Campynet protocol (http://campynet.vetinst.dk/PFGE.html) is routinely used by the EURL.
In 2011, the protocol developed by PulseNet (USA- PulseNet) (http://www.cdc.gov/PULSENET/protocols.htm) was compared with the Campynet protocol and both protocols are now established at the EURL.

In 2010, a 3-year research project "The distribution of putative virulence factors among *Campylobacter* isolates from poultry and humans" was initiated in collaboration with Uppsala University, Sweden. One of the project aims is to study occurrence of putative virulence factors in chicken and human *Campylobacter jejuni* strains of certain PGFE types. This project will be continued in 2012.

The MLST (Multilocus sequence type) method according to the protocol of Dingle et al. (J. Clin. Microbiology, 2001, 39: 14-23) was set up at the laboratory in 2010 and is used for studying populations of *Campylobacter jejuni*. Subtyping of the EU baseline survey (2008) isolates by PFGE and MLST will continue in 2012. The typing will provide information about prevalent strain types and degree of strain diversity among *C. jejuni* in broilers in Europe.

The EURL participates in a new national project "Attributing human cases of campylobacteriosis to their sources as a tool for targeted interventions". The project, which is financed by The Swedish Research Council Formas, includes collaboration between researchers from the veterinary, food and human sectors for collecting epidemiological data and strains. *Campylobacter* isolates will be characterized by MLST typing.

3.4. Participation in international networks and scientific meetings

In 2012, EURL staff members will participate in:

- MedVetNet Association. The European Network of Excellence MedVetNet ended in 2009 and an Association with the same name was formed. The EURL will participate in relevant activities of the new Association.

- ISO/CEN standardization activities:
  - Working group CEN/TC 275/WG 6/TAG 5, considering documents regarding *Campylobacter* in Primary Production (EN-ISO 10272 part 4) and Sampling techniques - Primary Production Stage.
  - Revision of ISO 10272 Part 1 and Part 2. The work started in 2009 and will continue in 2012

- Other relevant national and international seminars and research meetings in order to assure competence and knowledge on recent advancement within the *Campylobacter* area, for example FoodMicro2012 in Istanbul, Turkey

- As Member of the Advisory Board to the EU FP7 financed project "*Campylobacter* control – novel approaches in primary poultry production" (acronym: CamCon). Coordinator of project: Merete Hofshagen, National Veterinary Institute, Norway

- As leader for an Expert Group on Campylobacteriosis for the DISCONTOOLS project. The project is funded by the European Commission services and was started in 2008 (http://www.discontools.eu/home/index). The group has delivered the material that was needed for the database, but will continue to contribute with expert advice on *Campylobacter* to the project.

4. Assistance to the European Commission and the NRLs including ad hoc activities

The EURL will provide scientific and technical assistance to the Commission and the NRLs on issues regarding *Campylobacter*.

The EURL will collect information about the NRLs´ needs for laboratory training of molecular methods for identification and characterization of *Campylobacter*. Depending on the most urgent needs, relevant training activities will be organized in 2012.

EURL staff will continue to act as tutors at training programmes such as the Microbiology course for third countries within the European Training Platform for Safer Food Programme (DG SANCO).

Campylobacterisos is one of the diseases in focus for ECDC's Programme on Food and Waterborne Diseases and Zoonoses (FWD) at the annual meeting in October 2011. The EURL will collaborate with ECDC and provide assistance in the work with harmonizing surveillance including analytical methods for campylobacteriosis in humans.

Request from the NRLs and the Commission for support and advice will be handled by the EURL scientific staff as soon as possible. Assistance to the Commission and EFSA services will have priority.

5. Missions

The following missions are planned to be made in 2012 to:

- The 31st meeting of ISO/TC34/SC9 and the 19th meeting of CEN/TC275/WG6, which will be held in Brussels in May or June 2012. Total duration of the two meetings will be 5 days.

- One meeting with working group CEN/TC275/WG6 TAG 5 "Primary production stage", date not set yet. Duration is probably 2 days.

- For the revision of ISO 10272 standards, it is expected that 1 meeting will be organised. The meeting is suggested to be organised by the EURL in Uppsala in adjunct to the EURL workshop in 2012. In that case, there will be no travel, hotel, or subsistence costs for EURL staff.

- If requested, the EURL will visit NRLs- *Campylobacter* for assistance with performance of *Campylobacter* analyses.

- If relevant, 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.

- One co-ordination meeting of EURLs in the area of veterinary public health- biological risks, a meeting that is anticipated to be organized by DG SANCO in 2012.

- Participation in FoodMicro2012 in Istanbul, Turkey 3- 7 September, http://www.foodmicro2012.com/index.php

6. Communication

The EURL- *Campylobacter* webpage will be improved continuously in order to increase the utility for the NRLs. Information about analytical methods, proficiency tests, validation studies, and other relevant information will be made available in a user friendly style. The contents will be adjusted to the needs of the NRLs in their daily activities.
The QuestBack system will be tested for reporting PT results, responding to surveys (questionnaires) from the EURL and registration to PTs and workshops.

The EURL will continue to co-operate with EU Commission Services and other organisations and authorities working in the field of human and animal health.

7. Meetings.

A meeting with the ISO working group on revision of ISO standard 10272 will be organised by the EURL in 2012. The meeting is planned to take place on the day before the EURL-*Campylobacter* workshop in 2012, in Uppsala. A maximum of 10 active participants in the working group will be invited and reimbursed for travel and accommodation one night.

8. Training activities

A training course in the application of molecular techniques is planned to be organized for a maximum of 6 participants in 2012. The training course will either be focused on PCR for identification of thermophilic *Campylobacter* spp or PFGE technique for the characterization (typing) of *Campylobacter* (mainly *C. jejuni*). The contents of the course will be decided at the workshop in 2011, where needs for training activities will be discussed.