



REVISED Work Program (8 November 2012)

EURL- *CAMPYLOBACTER*

WORK PROGRAMME FOR 1st OF JANUARY 2013 TO 31st OF DECEMBER 2013

Introduction

The activities in the work programme for 2013 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. Performance indicators are included in a separate annex (Annex Performance Indicators)

The work programme for 2013 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 of methods for *Campylobacter* analysis
7. Communication

Activity 1

Organisation of Proficiency Tests, PTs, in 2013

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 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 10 proficiency tests for the NRLs. Three to five Official Laboratories (OLs) in third countries have been invited to participate in the PTs each year. Five 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.

In 2013, the EURL plans to organise two PTs, one of complexity grade 3, one of complexity grade 2. All EU NRLs are expected to participate. OLs in BA, CH, IS, MK and NO will be invited to participate. The PTs are described below.

It is expected that > 75% of the participating laboratories provide results that are graded as 'acceptable' or higher in both PTs.

For isolation, detection and identification of *Campylobacter* in PT 11, reference will be made to the SOP for the PT which in turn will basically follow the ISO 10272 standard. For PT 12, the NRLs and OLs can use the methods they find suitable (in house or reference methods). Both PTs will provide 10-12 samples including bacterial reference material and for PT 11 it will include relevant matrix (boot socks with chicken faeces).

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 (visit the NRL) or NRL staff will be offered to visit the EURL for training.

PT 11. Detection and species identification of *Campylobacter* in faeces material

The planned proficiency test number 11 will consist of detection and species identification of *Campylobacter* in chicken faeces material collected by sock sampling. The analysis will basically follow the ISO 10272 part 1: 2006 standard. Vials with freeze dried bacterial cultures will be used as reference material. The matrix (sock samples with faeces) will be collected from a *Campylobacter* free chicken farm and thoroughly tested to ensure freedom from contaminating *Campylobacter* before the test is distributed to the NRLs.

The EURL will prepare a standard operating procedure (SOP) for the PT and a Test report. The reporting of test results will be made by using an online service, QuestBack, which was tested in 2012. 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 2013.

The PT is planned to be distributed by courier in the spring 2013. Details about the PT and the exact date for distribution will be discussed with the NRLs at the workshop in October 2012.

PT 12. Detection and identification of *Campylobacter* in swab samples.

The planned PT number 12 will consist of swabs with bacterial cultures. Selected bacterial 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 should be cultured on agar plates and the bacterial growth should be analysed for *Campylobacter*. Species identification should be carried out. The NRLs can use any method for the identification (phenotyping and/or molecular methods). The EURL will provide instructions for handling of samples and the reporting of test results will be by QuestBack as for PT 11. The PT will be sent by courier in spring 2013, probably together with PT 11.

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 a new type of sample (matrix) and to validate and/or participate in validation studies of methods.

Validation of methods and reference materials for preparation of PT 11.

Detection and species identification of *Campylobacter* in chicken faecal material

Monitoring of *Campylobacter* in broiler flocks could be done by sock sampling, i.e. socks applied on the boots of a person who walks in the broiler house are submitted to the laboratory for analysis. This procedure has become common practice in many countries, but no “standard” method has been developed and validated. Furthermore, the EFSA biohazard panel report on harmonised epidemiological indicators for broiler meat inspection urged MSs to investigate the suitability of sock sampling for testing *Campylobacter*. For PT 11, the EURL plans to develop such a test. The EURL will prepare a “standard” sock with chicken faeces from a *Campylobacter* free broiler flock. The bacterial reference will be freeze-dried material (with *Campylobacter* and some blanks) as has been used in previous PTs. The EURL will test batches of the freeze dried reference material with the socks. The analysis of socks will basically follow the standard ISO 10272 part 1: 2006. Repeated tests will be made to ascertain the stability of the test material and 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 time to some destinations and the EURL therefore has to prepare samples that are stable for at least 4 days.

Participation in a validation study of ISO 10272.

The EURL will participate in a validation study of ISO 10272 Part 1 and Part 2:2006. The validation study will be organized by the Food and Consumer Product Safety Authority and National Institute for Public Health and the Environment, in The Netherlands. The study is planned to be performed in 2013. The EURL proficiency tests in 2011 and 2012 served as pre-studies for the validation.

Validation of a real-time PCR assay A real-time PCR assay, validated for detection of *Campylobacter* in poultry faeces on cloacae swabs will be validated with caecum samples. The results will be presented and discussed at the workshop in 2013.

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 2013

The EURL plans to organise the annual workshop in 2013 in adjunct to the international *Campylobacter*, *Helicobacter* and related organisms (CHRO) conference in Aberdeen, Scotland (<http://www.chro-2013.org/>). The CHRO conference starts 15 September and ends at noon on the 19th of September. The planned EURL- *Campylobacter* workshop will last from lunch – to – lunch from the 19th to 20th of September.

The plan is to invite representatives from the Member States' 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.

Since the workshop will be shorter than previous years (two half days instead of one and a half day), the agenda will be concentrated to focus on topics of immediate interest for the NRLs. But in general, 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 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

At least one NRL representative from each EU MS is expected to participate in the workshop in 2013. Actions taken to ensure participation include:

- The date for the workshop in 2013 workshop will be announced already at the workshop in 2012 (First announcement)
- A second announcement with details will be sent out about 4 months before 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.

3.2 EURL staff visits (missions) to NRLs for training of NRL staff

If an NRL has problems with the performance of *Campylobacter* analysis and needs assistance, the EURL will suggest a visit to the NRL for training of the staff.

One such visit (mission) is planned for 2013. Before the mission, the EURL staff will prepare laboratory material, relevant literature and presentation material needed for the visit.

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 6 participants in 2013. 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*. The contents of the course as well as needs for training activities will be discussed at the workshop in 2012.

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 session, the EURL will make preparations that include testing assays, bacterial strains, making laboratory protocols and lists of suppliers of reagents, chemicals, equipment, etc., and collect relevant literature for the participants/visitors.

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.

Provision of expertise to stakeholders

Request from the Commission and agencies for scientific and technical assistance will have priority and be handled by the EURL scientific staff as soon as possible.

One person of the EURL staff will continue to be a member of the EFSA Task Force on Zoonoses Data Collection in 2013.

On request, EURL staff will continue to 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:

- If *Campylobacter* is on the Agenda: 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 2013.

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, i.e.

- 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 DISCONTTOOLS 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.
- Other relevant national and international seminars and research meetings in order to assure competence and knowledge on recent advancement within the *Campylobacter* area, especially the conference “*Campylobacter*, *Helicobacter* and related Organisms (CHRO)”, 15 – 19 September 2013 in Aberdeen, UK, <http://www.chro-2013.org/>
- MedVetNet Association (MVNA). 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 MVNA.

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 will provide 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 person is active member of working groups:

- 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: 2006

The following meetings will be attended in 2013:

- The 32nd meeting of ISO/TC34/SC9 and the 20th meeting of CEN/TC275/WG6, which will be held in Berlin in June 2013. 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 in 2013.

Activity 6

Development of methods for *Campylobacter* analysis

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

Performance Indicator 6: To take account of scientific development activities at national and EU level and perform applied research and development activities whenever appropriate.

Objectives:

To achieve more knowledge and experience of methods for detection, identification and characterization of *Campylobacter* in order to provide the NRLs with details about the methods and advances in the field of *Campylobacter* analysis.

Methods for strain characterization of *Campylobacter*.

Strain characterization ‘subtyping’ of *Campylobacter* 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 recently been identified, much thanks to molecular (DNA-based) typing methods. Outbreaks due to consumption of raw milk in The Netherlands and Hungary and of consumption of raw peas in the US are examples of outbreaks which could be resolved by use of molecular subtyping methods (1, 2, 3).

There are methods and standardised protocols available through internet that could be recommended for use. It is expected that strain typing will be more often requested especially in outbreak situations but also in source attribution studies of sporadic cases, and the NRLs are increasingly asking for assistance with molecular subtyping methods. The EURL supports the use of Pulsed Field Gel Electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST) for strain typing.

To be prepared for outbreak situations, 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. The EURL tests modifications and adjustments of the protocols in order to optimize the methods and make them robust for the types of strains that are to be analysed.

Protocols used for strain characterization/subtyping of *Campylobacter*

PFGE: The standardised Campynet protocol (<http://campynet.vetinst.dk/PFGE.html>) is routinely used by the EURL. The protocol developed by PulseNet (USA- PulseNet) (<http://www.cdc.gov/PULSENET/protocols.htm>) has been compared with the Campynet protocol and both protocols are now established at the EURL.

MLST: The MLST method according to Dingle et al (2001) (4) has been established and the protocol is available at <http://pubmlst.org/campylobacter/>

The management and handling of PFGE and MLST typing data has been discussed in collaboration with Centers for Disease Control and Prevention (CDC) in United States.

The EURL collaborates with the Swedish NRL- *Campylobacter* in research projects that among other things include strain characterization by PFGE and MLST. 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.

Publications/communications

The publication plan for 2013 is that EURL staff co-authors 2 scientific publications in peer reviewed journals.

The EURL will participate in the CHRO conference in Aberdeen 2013 and contribute with oral and poster presentations.

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.

Most communication with NRLs, the Commission, and stakeholders is done by emails and consists of both short questions and more complicated issues, sometimes on ad hoc basis. Communication is also done by telephone and by the website and the QuestBack system. The QuestBack will be further tested for reporting PT results, responding to surveys (questionnaires) from the EURL and registration to PTs and workshops.

The time before and after workshops and PTs are the periods with most intensive contacts with NRLs. The EURL prepares reports of the PT results which are distributed to the NRLs and DG - SANCO by email. Communication by emails will continue but some types of messages will be transferred to the webpage which will be improved to increase the support to NRLs and stakeholders.

The work with preparation of work programme, budget forecast and technical and financial reports for submission to the Commission has become more detailed which leads to more hours being spent and more persons involved in these activities in 2013.

References

1. Heuvelink AE, et al. 2009. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. *Int. J. Food Microbiol.* 134, 70-74.
2. Kalman M, et al. 2000. Milkborne *Campylobacter* infection in Hungary. *J. Food Protect.* 63, 1426-1429.
3. Gardner TJ, et al 2011. Outbreak of campylobacteriosis associated with consumption of raw peas. *Clin. Infect. Dis.* 53, 26-32.
4. Dingle KE, et al. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. *J. Clin. Microbiology*, 39: 14-23.