TECHNICAL REPORT

Survey of National Reference Laboratory (NRL) capacity for six food- and waterborne diseases in EU/EEA countries

Campylobacteriosis, listeriosis, salmonellosis, Shiga toxin/verocytotoxin–producing *Escherichia coli* (STEC/VTEC), shigellosis and yersiniosis

www.ecdc.europa.eu

ECDC TECHNICAL REPORT

Survey of National Reference Laboratory (NRL) capacity for six food- and waterborne diseases in EU/EEA countries

Campylobacteriosis, listeriosis, salmonellosis, Shiga toxin/verocytotoxin–producing *Escherichia coli* (STEC/VTEC), shigellosis and yersiniosis

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Johanna Takkinen, and produced by Akke Vellinga and Martin Cormican from the National University of Ireland on behalf of the European Food- and Waterborne Diseases and Zoonoses programme, April 2012.

Authors:
Akke Vellinga (University of Ireland) and Martin Cormican (University of Ireland)

Acknowledgements:
We would like to thank all public health laboratories in the EU and EEA countries that responded to the survey. We also would like to express our gratitude to the following people for their significant contributions to the preparation of the report:
Tina Purnat, Eloise Valli, Therese Westrell, Nadia Ciampa, and Angela Lahuerta-Marin

Suggested citation: European Centre for Disease Prevention and Control. Survey of National Reference Laboratory (NRL) capacity for six food-and waterborne diseases in EU/EEA countries. Stockholm: ECDC; 2012.

Cover picture: Nathan Reading

Stockholm, July 2012

ISBN 978-92-9193-379-2

doi 10.2900/61436

Contents

Abbreviations iv
Summary 1
List of tables 2

1 Background and objectives 3
2 Methods 4
3 Results 6
4 *Salmonella* 7
5 *Campylobacter* 12
6 *Yersinia* 17
7 *Listeria monocytogenes* 22
8 STEC/VTEC 28
9 *Shigella* 34
10 Discussion 39

Conclusion 42
Bibliography 43
Annexes: Sample survey forms 44

Abbreviations

AFLP	Amplified fragment length polymorphism
AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-producing *Enterobacteriaceae*
EEA	European Economic Area
EIA	Enzyme immunoassay
EQA	External quality assessment
ESBL	Extended spectrum beta-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FWD	Food- and waterborne diseases
MLST	Multilocus sequence typing
MLVA	Multiple loci VNTR analysis
NRL	National reference laboratory
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
RFLP	Restriction fragment length polymorphism
STEC	Shiga toxin-producing *Escherichia coli*
VTEC	Verocytotoxin-producing *Escherichia coli*
WHO GSS	World Health Organization Global Salm Surv
WHO GFN	World Health Organization Global Foodborne Infections Network (former WHO GSS)

Summary

Data from National Reference Laboratories (NRLs) play an important part in European surveillance of food- and waterborne infectious disease. In 2009, a survey was conducted to obtain an idea of the availability and capacity of services offered by National Reference-level Laboratories in EU and EEA countries in relation to six priority food- and waterborne pathogens: *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, Shiga toxin/verotoxin–producing *Escherichia coli* (STEC/VTEC) and *Yersinia*. The survey was administered in the form of six questionnaires (i.e. one per pathogen) and sent to 118 contact points identified in the Member States on 2 July 2009. The deadline for returning the questionnaire to ECDC was 24 August 2009. The analyses were performed in 2010, and the interpretation of the findings started in January 2012.

The survey covered a number of topics: pathogen-specific methods in use in the NRLs, for (a) detection and confirmation, (b) further characterisation and (c) antimicrobial susceptibility testing (AST); participation in External Quality Assessment (EQA) schemes and relationship of the NRL with national institutes of health. There were also questions on training and needs in relation to EQA schemes and method standardisation/harmonisation. The overall response rate was high (80% or more) although no responses were received from any contact points for any/most pathogens in some countries and incomplete responses from others. Despite this limitation, the survey is the most comprehensive to date of reference laboratory capacity for six food- and waterborne diseases in Europe and provides a baseline for assessing this capacity and identifying gaps.

Designated NRL services are comparably most complete for *Salmonella*, even though there is considerable heterogeneity in the number and level of characterisation of the isolates. *Campylobacter*, the most common bacterial cause of diarrhoea, has a lack of designated NRLs in many countries, often concurrent with limited capacity to characterise (even to species level). STEC/VTEC detection and characterisation services are generally available but the characterisation varies from the ability to detect a single serotype (O157), without the capacity to confirm toxin gene, to the capacity to characterise all recognised variants of this complex group of pathogens. NRL services for *Listeria*, *Shigella* and *Yersinia* are similarly very diverse. Across all pathogens, antimicrobial susceptibility testing also varies widely from comprehensive in some laboratories to non-existent in others. For the emerging AMR threats amongst the *Enterobacteriaceae*, nearly all laboratories do susceptibility testing for one or more agents that would allow detection of extended spectrum beta-lactamases (ESBLs) but few are testing for agents that would allow detection of carbapenemase-producing *Enterobacteriaceae* (CPE).

Molecular methods for characterisation are available in most but not all laboratories for some or all pathogens. A working relationship with the national institute of public health is generally in place. This is important to ensure that the NRL services function as part of a real-time public health protection infrastructure and not as an isolated exercise. Quality management systems are in place in many laboratories in the form of accreditation according to ISO standards (17025 or 15189) and most laboratories have both internal and external quality control systems in place. The extent to which NRL services depend on ECDC and the Global Foodborne Infections Network (formerly Global Salm Surv) for specialised EQA is highlighted by the findings of the survey. Laboratories generally acknowledge the need for further training and harmonisation/standardisation of methods across Europe to improve their contribution to the protection of public health.

The most striking finding emerging from this survey is the lack of consistency in NRL capacity across Europe. Various European countries have little or no effective reference laboratory capacity to confirm or characterise some important food- and waterborne pathogens. In the context of European systems to support free movement of goods (food) and people, outbreaks of food-borne infectious disease or changing trends are European issues. However, at present, the capacity for laboratory detection and confirmation of outbreaks and changing trends in relation to food-borne infection is very weak across large parts of Europe. This divide in terms of NRL capacity represents a serious weakness in capacity for early detection and response. Although training and standardisation of methods and provision of external QA systems emerge from this survey as important supports that ECDC can provide to NRL services, the fundamental problem is the lack of laboratory capacity in some countries to support effective day-to-day public health action. This report highlights the urgent need to harmonise NRL services across Europe in terms of methods and to ensure the minimum operational capacity required to contribute to a Europe wide network for public health protection.

List of tables

Table 1: Summary of countries participating in the NRL survey in 2009. 6
Table 2: Overview of molecular typing methods for *Salmonella* spp. in 2012. 8
Table 3: Overview of methods and antimicrobial agents tested for *Salmonella* spp. in 2012. 10
Table 4: Overview of molecular typing methods for *Campylobacter* spp. in 2012. 13
Table 5: Overview of methods and antimicrobial agents tested for *Campylobacter* spp. in 2012. 15
Table 6: Overview of molecular typing methods for *Yersinia* spp. 18
Table 7: Overview of methods and antimicrobial agents tested for *Yersinia* spp. in 2012. 20
Table 8: Overview of molecular typing methods for *Listeria monocytogenes* in 2012. 24
Table 9: Overview of methods and antimicrobial agents tested for *Listeria monocytogenes* 26
Table 10: Overview of molecular typing methods for STEC/VTEC 30
Table 11: Overview of methods and antimicrobial agents tested for STEC/VTEC in 2012. 32
Table 12: Overview of molecular typing methods for *Shigella* spp. in 2012. 35
Table 13: Overview of methods and antimicrobial agents tested for *Shigella* spp. in 2012 37

1 Background and objectives

The aim of the survey questionnaire for the National Reference Laboratories (NRLs) was to achieve a better understanding of laboratory capacities for the six priority enteric pathogens (i.e. *Campylobacter, Listeria, Salmonella, Shigella,* STEC/VTEC and *Yersinia*) across Europe. This in turn would help to identify areas requiring public health laboratory capacity to be strengthened to improve the surveillance of these diseases.

The information gathered provides the basis for future ECDC work on strengthening detection, confirmation, and further characterisation of the six priority enteric pathogens. Furthermore, the survey has helped ECDC to assess the needs for existing and new EQA schemes, training and harmonisation of methods for these pathogens among EU Member States and EEA countries.

The survey was administered in the form of six questionnaires (i.e. one per pathogen) which aimed to assess the following:

- Pathogen-specific methods in use in the NRLs for (a) detection and confirmation, (b) further characterisation and (c) antimicrobial susceptibility testing (AST);
- Participation in External Quality Assessment (EQA) schemes and EQA scheme needs for further characterisation and AST;
- Laboratory accreditation;
- Training needs in detection and confirmation, further characterisation and AST and suggestions on how to best meet these needs;
- Method standardisation/harmonisation needs.

2 Methods

Survey development

The survey questionnaire was developed by the Food- and Waterborne Diseases and Zoonoses (FWD) programme in consultation with the experts in the FWD Network (nominated disease-specific experts in the Member States and EEA countries) and colleagues at ECDC.

To facilitate the comparison of the responses, the survey was developed using mostly closed-ended questions, with predefined codes per question or a scale for ranking preferences (1–5).

The survey was defined in the form of six questionnaires (i.e. one per pathogen), each of which consisted of seven sections: Introduction, Contact Information, General Information about the Laboratory, Methods used in the Laboratory, QC and EQA, Training Needs, and Harmonisation Needs. The survey forms were produced in separate Excel workbooks to be filled out and returned to ECDC by email before the specified closing date. The forms clearly marked the cells in which the responses for each question were to be recorded. These response cells used Excel cell validation to define the options that the respondent could use when answering the relevant question.

The plans for the survey were discussed with the FWD Network in October 2008. Various drafts of the survey were then circulated within the FWD Network and among ECDC colleagues. The survey was piloted in April 2009 with three laboratory experts. The final versions of the survey forms were produced in July 2009 (Annexes: Sample survey forms 1–6).

Survey administration

The target audience of the survey were national reference-level laboratories for the specific pathogens in the Member States and EEA countries. Therefore, the survey was sent to the disease-specific laboratory experts nominated by their countries to participate in the FWD Network. For those countries that had not nominated such experts when the survey was sent out, the ECDC National Contact Points for Surveillance were asked to give the names of experts in their country who could provide information for the survey. ECDC National Microbiological Focal Points (NMFPs) were also informed of the activity.

The survey was sent out to the 118 identified contact points in the Member States and EEA countries on 2 July 2009, followed by a reminder on 18 August 2009. The deadline for return of the questionnaire to ECDC was 24 August 2009.

Survey analysis

During March and April 2010, the replies from each country were compiled in one master Excel workbook per pathogen. Each dataset was cleaned for consistency and validity of coding for each question and each set of inter-related questions, and all free-text answers were coded into comparable summaries, where the context allowed. All changes to coding were recorded in an analysis log.

A first draft report was received by ECDC on 20 January 2012, after which additional information became available and changes were made. A draft for consultation was sent to contact points in the countries during February 2012 with a closing date for comments of 2 March. Additional information provided by contact points up to that date was included in the report. During the project, the name of WHO Global Salm Surv (WHO GSS) changed to WHO Global Food-borne Infections Network (WHO GFN) and this name is used instead of WHO GSS throughout the report.

Completeness of data and limitations

When examining answers to detailed sub-questions it became clear that there were some inconsistencies due to different interpretations of the questions. For example, a response may have indicated that no further typing of *Salmonella* was performed (Q33 of *Salmonella* questionnaire) but a subsequent answer (Q410-426c) indicated that serotyping and molecular typing were carried out. Where such inconsistencies were obvious and easily corrected this was taken into account in the analysis. Inconsistencies also appeared in terms of incomplete answers to a small number of questions. For example, in all six questionnaires it became apparent that questions 331A and 331B (Please provide information on clinical sample volumes in 2008 by replying either to A or B) were interpreted so differently that the information was of no value. On this basis replies to a small number of questions were excluded from the report. For future surveys it would be useful to revise some of the questions to minimise ambiguity.

For the purpose of this report a reference laboratory is understood to be a laboratory that accepts samples and cultures of bacteria isolated in other laboratories for confirmation and/or further characterisation. Reference laboratories may or may not perform primary isolation of pathogens from clinical samples. In some cases

respondents indicated that there was no designated national reference laboratory for a specific pathogen and that for this reason they returned the questionnaire without detailed responses. These essentially blank questionnaires were not included in the analysis although the summary (Table 1) indicates that a response was received. Some respondents (for example Malta for all six pathogens) indicated that, although there is a laboratory that performs a degree of isolate identification and characterisation similar to that provided by some reference laboratories, there is no designated reference laboratory and they do not accept isolates from other laboratories. For the purposes of this report all detailed responses were included in the analysis even where the respondent indicated that they are not a designated reference laboratory for human clinical isolates/samples.

In the report, the country where the laboratory is based is sometimes used as a synonym when referring to the laboratory, in a similar manner to the way in which the term 'respondent' is used throughout the document.

There appears to be no national institute of (public) health in Malta and Cyprus and their answers to the questions referring to the working relationship with such a body must be considered in this context.

The report provides the most comprehensive overview available to date regarding the availability and quality of reference laboratory services in the EU and EEA/EFTA for six priority food- and waterborne human pathogens in the EU Member States and EEA countries, as of August 2009. Some elements (relating to molecular typing methods and antimicrobial susceptibility testing) were updated in 2012.

3 Results

Response rates

The response rate for all six pathogens was 80% or higher (Table 1). Portugal, Latvia and Liechtenstein are not included in this report because no replies were received from laboratories in these countries in relation to any pathogen.

Table 1: Summary of countries participating in the NRL survey in 2009

	Salmonella	*Campylobacter*	*Yersinia*	*Listeria*	VTEC[1]	*Shigella*
Total	**N=26**	**N=25**	**N=24**	**N=25**	**N=25**	**N=25**
Response rate	**87%**	**83%**	**80%**	**83%**	**83%**	**83%**
EU						
Austria	X	X	x	x	X	X
Belgium	X	X	X	X	X	X
Bulgaria	X	X	X	X	X	X
Cyprus	X	X	X	X	X	X
Czech Republic	X	X	X	X	X	X
Denmark	X	X	X	X	X	X
Estonia	X	X	X	X	X	X
Finland	X	X	X	X	X	X
France	X	X	X	X	X	X
Germany	X	X	X	X	X	X
Greece	X	X		X	X	X
Hungary	X	X	X	X	X	X
Ireland	X	X	X	X	X	X
Italy	X	X	X	X	X	X
Latvia						
Lithuania	X	X	X	X	X	X
Luxembourg	X	X	X	X	X	X
Malta	X	X	X	X	X	X
Netherlands	X		X	X	X	
Poland	X	X	X	X	X	X
Portugal						
Romania	X	X	X	X	X	X
Slovakia	X	X				X
Slovenia	X	X	X	X	X	X
Spain	X	X	X	X	X	X
Sweden	X	X	X		X	X
United Kingdom				X		
EEA						
Iceland	X	X	X	X	X	X
Liechtenstein						
Norway	X	X	X	X	X	X

[1] Verocytotoxin-producing *Escherichia coli*

4 *Salmonella*

Responses were obtained from laboratories in 24 EU and two EEA countries. No response was obtained from Latvia, Portugal, the United Kingdom or Liechtenstein. Some laboratories did not respond to all questions, therefore the total number of responses is not 26 for all questions.

Reference laboratory services

All of the 26 laboratories that responded were involved in giving microbiological advice and support during outbreak investigations.

In relation to direct sample/isolate processing most respondents provide further typing (25), antimicrobial susceptibility testing (24), primary isolation (14) and non-culture based identification (14). All laboratories, with the exception of the one in Malta, maintain a strain collection. Many laboratories (14) indicate that they receive all isolates while laboratories in the Czech Republic, the Netherlands and Italy reported a structured sampling plan.

Among the 25 laboratories that maintain strain collections, in 22 of them the collections consist of all the isolates sent in, while the other laboratories only store samples related to an outbreak and/or on request.

Laboratories provide a wide range of support services with most of them providing training (18), microbiological advice (26), method development (19), research and scientific publication (20), support in outbreak investigation (26), and guidelines on diagnostic procedures for primary laboratories in their own country (14). Some laboratories are involved in organising ring trials (10), providing material for proficiency testing organisers (7), supporting quality assurance in primary laboratories (15) and providing reference material to primary laboratories (13).

The majority (18 out of the 26 laboratories) also process *Salmonella* from food and 13 also from animals.

The laboratory is part of the national institute for public health in Bulgaria, Czech Republic, Denmark, Finland, Luxembourg, Norway, Poland, Slovakia, and Sweden and another 15 laboratories report a working relationship with their national public health institute. The remaining two (Cyprus and Malta) do not have a national public health institute. Sixteen laboratories collaborate with their national public health institute in the area of surveillance, outbreak investigation and typing. Meanwhile 12 of them also collaborate on research activities.

The volume of human *Salmonella* isolates received by the laboratories varied widely between 48 (Poland) and 7 439 (France) with an average of 1 700 and a median number of 900. Only one laboratory received less than 100 isolates per year, three between 100 and 300 and the other 22 more then 300.

Methods

All 26 laboratories use culture methods to identify and confirm *Salmonella* and 16 use additional non-culture-based methods.

The most often mentioned routinely used selective media to culture *Salmonella* are XLD (11), Selenite broth (8), Hektoen (6), and *Salmonella-Shigella* agar (6), but a variety of other media are used by different countries. Further characterisation of the isolates beyond species level is routinely (19) or occasionally (5) performed.

All 26 laboratories use phenotypic methods to characterise isolates to species level and for further typing. All O and H antigen serotyping is performed by 23 laboratories and phage typing is performed routinely in 11 laboratories, and occasionally in other laboratories. PCR of the invA gene is used to some degree for species confirmation by a number of laboratories.

Typing by PFGE (Pulsed field gel electrophoresis) is performed in 23 laboratories and Multiple Loci VNTR Analysis (MLVA) in 13 laboratories (Table 2). Virulence gene detection (8) is performed occasionally. A number of other molecular typing methods are used, including Multi Locus Sequence Typing (MLST) and plasmid profiling. The seven laboratories that planned to implement a new method in the near future all intended to implement MLVA.

Table 2: Overview of molecular typing methods for *Salmonella* spp. in 2012

	PFGE	Multiple Loci VNTR	Ribotyping	Plasmid profiling
	N=23	N=13	N=2	N=2
EU				
Austria	x	x		
Belgium	x			
Bulgaria	x			
Cyprus				
Czech Republic	x			
Denmark	x	x		
Estonia	x			
Finland	x	x		
France	x	x	x	
Germany	x	x	x	
Greece	x			
Hungary	x			x
Ireland	x	x		x
Italy	x	x		
Latvia				
Lithuania				
Luxembourg	x	x		
Malta				
Netherlands	x	x		
Poland	x			
Portugal				
Romania	x	x		
Slovakia	x			
Slovenia	x			
Spain	x	x		
Sweden	x	x		
United Kingdom				
Non EU				
Iceland	x			
Liechtenstein				
Norway	x	x		

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) is performed routinely in the laboratories in 20 countries and occasionally in four countries (Bulgaria, Czech Republic, France and Poland). Only the laboratories in Sweden and Netherlands report that they do not perform AST. In 11 out of the 26 countries, a national surveillance programme for *Salmonella* antimicrobial resistance is in place.

AST is performed on all isolates in 13 laboratories and only on a selection in the others. Some countries only perform AST during outbreak investigation (16), or on a selection of isolates (six laboratories) or for specific research studies or projects (16 laboratories).

A variety of methods for AST are reported; most often disc diffusion (21). A total of 14 laboratories use a gradient strip MIC method regularly or occasionally and ten laboratories use dilution methods.

For interpretation Clinical and Laboratory Standards Institute (CLSI) criteria are the most widely applied (20), two laboratories also apply EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria and four apply national breakpoint interpretive criteria. Some laboratories report applying more than one method of measurement and interpretation for AST.

On average susceptibility to 13 antimicrobials is tested, with a minimum of five (Cyprus) and a maximum of 31 (France). Ciprofloxacin is always part of the panel, as is trimethoprim either alone or in combination with sulphamethoxazole (co-trimoxazole) (Table 3). An aminopenicillin (ampicillin in 23 and amoxicillin in France) is included in all laboratories and all but one (Slovakia) are testing for susceptibility to cefotaxime or ceftriaxone (indicating some capacity to detect extended spectrum beta-lactamase producers (ESBLs). Only four laboratories are testing for susceptibility to any carbapenem (relevant to detection of carbapenemase producers) and only six for susceptibility to a macrolide (6). Most commonly tested other antimicrobials are tetracycline (21), chloramphenicol (21), gentamicin (21), kanamycin (15) and streptomycin (13).

Twelve countries also identify resistance genes in *Salmonella* spp using molecular techniques.

For epidemiological surveillance of AST for *Salmonella* at EU level, the highest ranked molecular typing methods are PFGE and MLVA. Both methods are also mentioned in the need for harmonisation of methods at EU level.

Table 3: Overview of methods and antimicrobial agents tested for *Salmonella* spp. in 2012

CLASS		Penicillins			Cephalosporins*		Carbapenems	Quinolones†	Aminoglycosides		Sulfonamides‡	Chloramphenicol	Tetracyclines	Macrolides
Antimicrobial agent		Aminopenicillin§	Amoxicillin-clavulanic acid	Other	Second	Third	Imipenem		Gentamicin	Other				Erythromycin
	Method	N=24	N=11	N=5	N=4	N=23	N=4	N=24	N=21	N=21	N=24	N=21	N=21	N=6
EU														
Austria[1]	D,G	x				x		x	x	x	x	x	x	
Belgium[1]	D	x	x			x		x	x	x	x	x	x	x
Bulgaria[1]	C,D,G	x	x		x	x		x	x		x	x	x	
Cyprus[1]	C,D	x				x		x			x			
Czech Republic[1]	C,D	x	x			x		x	x	x	x	x	x	x
Denmark[2]	C	x	x	x		x		x	x	x	x	x	x	x
Estonia[1]	D,G	x				x		x	x	x	x	x	x	
Finland[1]	D,G	x		x		x	x	x	x	x	x	x	x	
France[3]	D,G	x	x	x	x	x	x	x	x	x	x	x	x	x
Germany[4]	C	x				x		x	x	x	x	x	x	
Greece[1]	C,D,G	x	x			x		x	x	x	x	x	x	x
Hungary[1]	D,G	x				x		x	x	x	x	x	x	
Ireland[1]	C,D,G	x				x		x	x	x	x		x	x
Italy[1]	C,D,G	x			x	x		x	x	x	x	x	x	
Latvia[1]														
Lithuania[1]	D	x				x		x	x	x	x	x	x	
Luxembourg[1]	D	x	x	x		x		x	x	x	x	x	x	
Malta[1]	G	x	x	x		x	x	x	x	x	x			
Netherlands[1]														
Poland[1,2]	C,D	x	x			x		x	x	x	x	x	x	
Portugal														
Romania[1]	D,G	x	x		x	x	x	x	x	x	x	x	x	
Slovakia[1]	C,D,G	x						x	x	x	x	x	x	
Slovenia[1]	D,G	x				x		x	x	x	x	x	x	
Spain[1]	D,G	x	x			x		x	x	x	x	x	x	
Sweden[1]														
UK														
Non EU														
Iceland[1]	D	x				X		x			x	x		
Liechtenstein														
Norway[5]	D	x				X		x		x	x	x	x	

**Luxembourg also tested for cefepime*
† Nalidixic acid/ciprofloxacin/ofloxacin
‡ Sulphonamide/trimethoprim/trimethroprim & sulphomethoxazole
§ Amoxicillin/ampicillin

[1] CLSI
[2] EUCAST
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)
[4] Deutsche Industrie Norm (DIN)
[5] Norwegian Working Group on Antimicrobials (NWGA)

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

Quality control

Most laboratories have internal quality control (IQC) systems in place and participate in regular external quality assessment programmes or inter-laboratory comparisons for some or all of their activities. Internal quality control programmes usually involve serotyping (19) and AST (19), but may also include identification/confirmation (16), species determination (17) and, less often, phage typing (10). Four laboratories report that they do not have IQC in place for any or their activities.

All laboratories are involved in EQA programmes for serotyping (26) and most (23) also carry out AST and species determination (17). Fourteen laboratories participate in isolation and identification EQA schemes and 12 in phage typing schemes.

The main schemes for external quality assessment were the ECDC scheme (19) and the WHO GFN EQA scheme (13).

Half (13) of the laboratories indicate that they are accredited for some or all of their services. Ten laboratories indicate that they are accredited to the ISO 17025 standard and three to the ISO 15189 standard (two laboratories have both). In addition, five countries were in the process of obtaining (additional) accreditation to the ISO 15189 standard.

Training

All but three of the laboratories report regular training activities for their staff in some or all of the following areas: quality control (23), typing methods (21), AST methods (20), bio-safety (20), and, to a lesser degree, in identification and confirmation (17) and accreditation (15).

The identified training needs in the laboratories mainly relate to typing methods, which are ranked highly by all laboratories. Training in AST, bio-safety, quality control and assurance, and accreditation are ranked lower. Training in identification and confirmation is ranked lowest in priority for all laboratories. The most appropriate way to address these training needs is felt to be in the form of hands-on training or short courses. Online training is less often considered to be the appropriate way of addressing the training needs.

Harmonisation

Most countries follow international recommendations for AST and indicate that they follow national or international recommendations/guidelines for some or all of their other activities in relation to further characterisation.

The NRLs provide training (3), guidelines (2) or both (10) to other primary laboratories in their country. This includes training/guidelines in all areas but mainly concerns identification/confirmation or typing methods. Some laboratories provide reference services to other countries for further characterisation (10), confirmation (8), isolation (4) and AST (4). Some countries request phage typing and identification/confirmation of new, novel or rare types of isolate from the Robert Koch Institute, Germany, the Pasteur Institute in France or the Health Protection Agency in the UK.

When harmonising methods at the EU level, most weight is given to serotyping, AST methods and phage typing. Other methods rank lower in the requirements to harmonise, although PFGE and MLVA typing are mentioned by a number of laboratories as additional methods that require EU level harmonisation.

5 *Campylobacter*

Detailed responses were obtained from 22 EU and two EEA countries. Ireland indicated that it has no reference laboratory service. Responses from Malta, Slovakia and Slovenia indicated that there is no designated reference laboratory service for *Campylobacter*, however some detailed information was provided from a laboratory with relevant expertise. No response was obtained from Latvia, the Netherlands, Portugal, the United Kingdom or Liechtenstein. Some laboratories did not respond to all questions which is why the total number of responses is not 24 for all questions.

Reference laboratory services

From the 24 laboratories providing detailed responses, three indicated that all human isolates are sent to their laboratory (Luxembourg, Austria and Iceland) and France receives all isolates from a series of sentinel laboratories. Four countries (Denmark, Norway, Slovenia and Slovakia) indicated that they receive a defined proportion of isolates. All laboratories provide support in outbreak situations with the exception of those in Bulgaria, Malta and Sweden. Less than half of the laboratories process *Campylobacter* from food samples (11 out of 24) and eight out of 24 laboratories process animal samples.

With regard to direct sample/isolate processing most respondents provide primary isolation (n=18), non-culture based identification (n=19), further typing (n = 19) and antimicrobial susceptibility testing (n = 21). All laboratories maintain a strain collection except for those in Finland and Malta. Of the 19 laboratories that routinely store isolates, 13 store all (confirmed) isolates, the laboratories in Sweden and Hungary store for study or project reasons and in Estonia some randomly selected isolates are stored.

The laboratories provide a wide range of support services with most laboratories providing training (14), microbiological advice (19) method development (15), research and scientific publication (20), support for outbreak investigation (23) and guidelines on diagnostic procedures for primary laboratories in their own country (14). Some laboratories are involved in organising ring trials (4), providing material for proficiency testing organisers (3), supporting quality assessment in primary laboratories (12) and providing reference material to primary laboratories (10).

In ten countries (Bulgaria, Czech Republic, Denmark, Finland, Hungary, Italy, Luxembourg, Norway, Poland and Slovenia) the NRLs are situated within the national institutes of public health. Twelve other laboratories indicated close collaboration between reference laboratory services and public health agencies. For most of the laboratories this collaboration covers support during outbreak investigations and surveillance.

The volume of human *Campylobacter* isolates received by the laboratories in 2008 varied widely from two (Romania) to 2 600 (Austria), with an average of 427 and a median isolate number of 260. Eight laboratories received less than 100 isolates per year.

Methods

The laboratories generally use culture methods to identify *Campylobacter* (23 out of 24 answers). A total of 22 laboratories indicated that they perform species-level identification on human isolates. The exceptions are Hungary and Spain. Non-culture methods (PCR-based) are used for identification by 17 laboratories. In Finland only non-culture methods are in use for confirmation of species.

Amongst laboratories that culture for *Campylobacter* spp., 15 list Charcoal Cefoperazone Desoxycholate Agar (CCDA) as a routinely used selective medium. A small number of laboratories use Skirrows, Butzler or Karmali's medium.

Further characterisation of isolates beyond species level is routinely (19) or occasionally (5) performed in all laboratories. Species determination by phenotyping is routinely offered in 17 countries and serotyping is performed in five countries. Molecular typing is performed by 15 laboratories (routinely in Finland, Norway, Hungary and Spain and occasionally in others). PFGE is the most widely used method, with some laboratories also using fla-PCR (Denmark, Greece, Luxembourg, Poland, Romania and Spain) and MLST (Denmark, Germany, Luxembourg, Poland and Spain) (Table 4). Of the laboratories that plan to introduce a new typing method MLST was the method being most widely considered (6).

Table 4: Overview of molecular typing methods for *Campylobacter* spp. in 2012

	PFGE	fla-PCR	MLST	AFLP	Other
	N=12	N=6	N=5	N=2	N=3
EU					
Austria	x				x
Belgium				x	x
Bulgaria					
Cyprus					
Czech Republic					
Denmark	x		x		
Estonia					
Finland	x				
France					
Germany	x	x	x		
Greece	x	x		x	
Hungary	x				
Ireland					
Italy	x				
Latvia					
Lithuania					
Luxembourg		x	x		
Malta					
Netherlands					
Poland	x	x	x		
Portugal					
Romania		x			
Slovakia					
Slovenia	x				
Spain	x	x	x		
Sweden	x				
United Kingdom					
Non EU					
Iceland	x				
Liechtenstein					
Norway					x

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) for *Campylobacter* spp. is performed by 21 out of the 24 laboratories with most (19) performing AST on a routine basis. AST is generally performed on all isolates, but sometimes on a set number or for specific research studies or projects. A variety of AST methods are reported, with 15 laboratories using gradient strip MIC method[2] regularly or occasionally. Disc diffusion (13) and broth dilution (9) are also used. For interpretation purposes, CLSI criteria are the most widely used (15) with three laboratories using EUCAST criteria and three using national breakpoint interpretive criteria. Some laboratories report using more than one method of measurement and interpretation.

The number of antimicrobial agents tested in each laboratory ranges from two to 14, with an average of seven antimicrobials tested. Susceptibility to ciprofloxacin and erythromycin are assessed in all laboratories and testing for susceptibility to nalidixic acid (18), gentamicin (17), tetracycline (16) and ampicillin (14) are also common (Table 5). A number of laboratories test susceptibility to agents for which no interpretive criteria or quality control criteria are specified by CLSI or EUCAST.

Five laboratories occasionally use molecular techniques to identify antibiotic resistance genes (Czech Republic, Hungary, Italy, Poland and Spain).

A national surveillance programme exists for AST of *Campylobacter* in nine out of 24 countries (Austria, Denmark, Estonia, France, Iceland, Italy, Norway, Poland and Slovenia).

[2] The most widely used gradient strip MIC method is E-test™ although the M.I.C.Evaluator™ is a similar technology.

Table 5: Overview of methods and antimicrobial agents tested for Campylobacter spp. in 2012

CLASS		Penicillins			Cephalosporins		Carbapenems	Quinolones*	Aminoglycosides		Chloramphenicol	Tetracyclines	Macrolides
Antimicrobial agent		Aminopenicillin†	Amoxyclav	Other	First	Third	Imipenem		Gentamicin	Other			Erythromycin
	Method	N=14	N=11	N=1	N=3	N=6	N=3	N=21	N=17	N=3	N=8	N=16	N=21
EU													
Austria[1,2]	C,D,G	x	x				x	x	x	x	x	x	x
Belgium[9]	C,D,G	x	x				x	x	x			x	x
Bulgaria[1]	C	x	x			x		x	x			x	x
Cyprus													
Czech Republic[1,3]	D,G					x		x	x		x	x	x
Denmark[1]	C							x	x	x	x	x	x
Estonia[3]	D,G	x	x					x	x			x	x
Finland[1]													
France[1]	D	x	x					x	x			x	x
Germany[1,5]	C	x		x		x		x	x	x	x	x	x
Greece[1]	D,G	x	x			x	x	x	x			x	x
Hungary	C,G							x					x
Ireland													
Italy[2]	D,G	x				x		x	x		x	x	x
Latvia													
Lithuania[2,6]	D,G							x					x
Luxembourg[1]	D,G	x	x		x			x	x		x	x	x
Malta[1,7]	G							x					x
Netherlands													
Poland[1]	C,G	x						x	x				x
Portugal													
Romania[1,3]	C,D	x	x		x	x		x	x		x	x	x
Slovakia[9]	D	x	x					x	x			x	x
Slovenia[1,3]	C,D,G	x	x		x			x	x			x	x
Spain[1]	D,G	x	x					x	x		x	x	x
Sweden													
UK													
Non EU													
Iceland[1]	G							x					x
Liechtenstein													
Norway[8]	G							x	x			x	x

** Nalidixic acid/ciprofloxacin/ofloxacin*
† Amoxicillin/ampicillin

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

[1] CLSI
[2] EUCAST
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)
[4] Swedish Reference Group for Antibiotics (SRGA-M)
[5] Deutsche Industrie Norm (DIN)
[6] British Society for Antimicrobial Chemotherapy (BSAC)
[7] Health Protection Agency (HPA)
[8] Norwegian Working Group on Antimicrobials (NWGA)
[9] No answer

Quality control

Fifteen out of the 24 laboratories report participation in regular EQA programmes for some or all of their activities including AST (11). Some laboratories cite the EQA programmes and include general EQA programmes (UK-NEQAS) for identification and/or susceptibility testing or programmes for EQA testing of other pathogens (e.g. *Salmonella* WHO GSS, nowadays known as WHO Global Foodborne Infections Network). Most laboratories have internal quality control for some or all of their activities (12) but nine reported no internal quality control in any area specified and three laboratories left the question unanswered.

Of the 15 laboratories that report having accreditation, eight laboratories have accreditation according to ISO 17025 and two have accreditation to both the ISO 17025 and ISO 15189 standards. Three other laboratories were pursuing accreditation at the time of completing the questionnaire.

Training

Most laboratories have staff training for some or all of their activities but nine of them indicated that they have no staff training activities in any of the areas specified and one left the question unanswered.

Amongst those areas in which training is provided, 15 of the 24 laboratories have regular bio-safety training, 14 provide training in identification and confirmation methods and 13 in typing, AST and quality control/assurance. When asked to rank their training needs, the laboratories gave the highest rank to typing methods, followed by quality control and AST methods.

Harmonisation

The laboratories indicate that national and/or international recommendations are generally implemented for AST and, to a lesser extent, for isolation, confirmation and species determination. Information regarding the specific recommendations followed was only collected for AST.

Fourteen laboratories provide guidelines and procedures for *Campylobacter* to primary laboratories in their own country. These methods often include identification/confirmation (13) and AST methods (12) and less often typing methods (6). In addition, some laboratories provide training in bio-safety (3), quality control procedures (4) and accreditation (2). Guidance and training are generally not provided for other countries, with the exception of Belgium, Czech Republic, Italy, Finland and France who provide some of these services. Belgium and Norway indicate they use some of the services provided by other countries.

The priorities for harmonisation of methods across Europe identified by most laboratories are AST and species determination and, to a lesser extent, harmonisation of methods for isolation and confirmation.

Among the typing methods identified as useful for surveillance purposes at EU level, MLST is ranked as the most suitable method. PFGE and *fla*-PCR were given lower rankings. In general, routine typing of *Campylobacter* is not felt to be a high priority, but a protocol for method harmonisation in outbreak situations would be of interest.

6 *Yersinia*

Responses were received from 22 laboratories in EU and two EEA countries. Ireland and Malta have no reference laboratory service. No replies were obtained from Greece, Latvia, Portugal, Slovakia, the UK or Liechtenstein. Some laboratories did not respond to all questions which is why the total number of responses is not 23 for all questions.

Reference laboratory services

Of the 22 laboratories providing detailed responses, six indicate that they receive all human isolates (France, Iceland, Italy, Austria, Luxembourg and Norway), while the other laboratories only receive isolates during outbreaks. Most laboratories that do not accept all isolates will accept isolates for diagnostic reasons in addition to outbreak situations.

Few laboratories process *Yersinia* isolates from animals (5). A total of 10 laboratories process food isolates.

As regards direct sample/isolate processing in five key areas, most respondents provide primary isolation (14), non-culture based identification (11), further typing (18) and antimicrobial susceptibility testing (16). Nearly all laboratories (19) maintain a strain collection, with the exception of Bulgaria, Estonia and Lithuania.

Of the 19 laboratories that store isolates, eight store all strains, and the others store all outbreak-related strains (8) and/or when specifically requested (10).

The laboratories provide a wide range of support services with many of them providing training (10), microbiological advice (20) method development (13), research and scientific publication (13), support in outbreak investigation (20) and guidelines on diagnostic procedures for primary laboratories in their own country (12). Some laboratories are involved in organising ring trials (4), providing material for proficiency testing organisers (7), supporting quality assurance in primary laboratories (7) and providing reference material to primary laboratories (7).

Nine laboratories are part of the national public health institute structure in their country (Bulgaria, Denmark, Finland, Italy, Lithuania, Luxembourg, Netherlands, Romania and Spain) with ten of the remaining laboratories indicating that they collaborate with their national public health institute. This collaboration usually involves outbreak investigations (13), surveillance (10) and typing (8) and to a lesser extent research (5). Three laboratories indicate no collaboration with their national public health institute and two provide no information on this issue.

The volume of human *Yersinia* isolates examined varies between zero (Cyprus and Hungary) and around 400 (Belgium and Finland). Most laboratories (12) examine less than 100 and six examine between 100 and 200.

Human isolates are categorised into *Y. enterocolitica* (generally more) and *Y. pseudotuberculosis* (few). The range for *Y.enterocolitica* is from 0 to 391. Between 200 and 391 human *Y. enterocolitica* isolates were examined in Belgium, France and Lithuania. In 2008, the laboratories in Austria, Germany, Norway, Poland and Spain received between 100 and 200 isolates while Denmark and Sweden received around 50. The remaining laboratories received less than 50 isolates. Human isolates of *Y. pseudotuberculosis* are examined in some countries and the number of isolates ranges from two to 28 (Poland 2, Austria 3, Sweden 4, Belgium 6, France 11 and Finland 28). Very few laboratories provide or use reference services from other countries. Only Belgium and France provide some reference services and Bulgaria and Norway sometimes use other laboratories for reference services.

Methods

The laboratories generally use culture methods to identify *Yersinia* (20) and additional non culture methods are used in ten laboratories. The Netherlands did not respond to these questions.

The selective media most widely used for culture is *Yersinia* selective agar (CIN), which was recorded by ten countries.

Phenotypic methods for characterisation are used by 22 laboratories for species determination (22), serotyping (20) and bio- typing (15 routinely and two occasionally). France also performs phage typing on a routine basis.

Molecular typing is occasionally carried out in nine and routinely in two countries. PFGE is most widely used (7), MLVA in four laboratories (Bulgaria, Finland, Norway, Poland) and MLST sometimes in Spain (Table 6). France occasionally performs ribotyping (RFLP).

Virulence gene detection is performed in ten countries (Austria, Finland, France, Germany, Hungary, Italy, Netherland, Poland, Slovenia and Sweden), four of which perform this routinely.

Further characterisation is generally performed when specifically requested and/or in outbreak investigations.

Table 6: Overview of molecular typing methods for *Yersinia* spp. in 2012

	PFGE	MLVA	Ribotyping
	N=7	N=4	N=1
EU			
Austria			
Belgium			
Bulgaria		x	
Cyprus			
Czech Republic			
Denmark	x		
Estonia			
Finland	x	x	
France	x		x
Germany	x		
Greece			
Hungary			
Ireland			
Italy			
Latvia			
Lithuania			
Luxembourg			
Malta			
Netherlands			
Poland	x	x	
Portugal			
Romania			
Slovakia			
Slovenia	x		
Spain	x		
Sweden			
United Kingdom			
Non EU			
Iceland			
Liechtenstein			
Norway		x	

Antimicrobial susceptibility testing

AST is performed in 16 laboratories and in 13 of these on a routine basis. AST is performed on all isolates in five countries; Austria, France, Iceland, Lithuania and Norway. Other countries only perform AST during outbreak investigations or when specifically requested by the laboratory that sends the sample. Of the 16 countries which perform AST, disc diffusion is the most frequent method used (13 using it routinely and one occasionally). Dilution methods are used by three countries and three countries occasionally use a gradient strip MIC method.

The 16 countries that perform AST include between three and18 antibiotics in their testing, with an average of 10. AST generally includes ciprofloxacin (15) and trimethoprim (14, with or without sulphamethoxazole) and tetracycline (15) (Table 7). Aminopenicillin (ampicillin or amoxicillin) and/or amoxicillin–clavulanic acid are usually also included. Other agents frequently included are chloramphenical (12), nalidixic acid (11), gentamicin (11), cefotaxime (8), streptomycin (6), imipenem (5), sulphonamide (4), cefoxitin (4) and kanamycin (4).

Most (14) of the laboratories report using the CLSI guidelines to determine breakpoints/cut-off values.

Molecular techniques to identify antibiotic resistance genes in *Yersinia* are occasionally used in France and Spain but in no other country.

Three countries have a national surveillance programme for antimicrobial resistance in *Yersinia* spp. (Austria, Iceland and Norway).

Table 7: Overview of methods and antimicrobial agents tested for *Yersinia* spp. in 2012

CLASS		Penicillins			Cephalosporins*†		Carbapenems	Quinolones‡	Aminoglycosides		Sulfonamides§	Chloramphenicol	Tetracyclines	Macrolides**
Antimicrobial agent		Aminopenicillins††	Amoxicillin-clavulanic acid	Other#	Second	Third	Imipenem		Gentamicin	Other§§				Erythromycin
	Method	N=13	N=7	N=4	N=6	N=12	N=5	N=15	N=11	N=9	N=14	N=12	N=15	N=4
EU														
Austria[1]	D	x							x	x	x	x	x	x
Belgium														
Bulgaria[1]	D	x	x		x	x		x	x	x	x	x	x	
Cyprus[1]	C	x	x	x	x	x	x	x	x		x		x	
Czech Republic														
Denmark														
Estonia[1,2]	D	x				x		x			x		x	
Finland														
France[3]	C,D	x	x	x	x	x		x			x		x	
Germany[1,4]	C				x	x	x	x		x	x	x	x	x
Greece														
Hungary[1]	D	x				x		x			x	x	x	
Ireland														
Italy[1]	D,G							x	x	x		x	x	x
Latvia														
Lithuania[1]	D							x	x		x	x	x	
Luxembourg[1,2]	D	x	x	x		x		x	x	x	x	x	x	
Malta														
Netherlands														
Poland[1]	D	x						x	x	x	x	x	x	x
Portugal														
Romania[1]	D,G	x	x		x	x	x	x	x	x	x	x	x	
Slovakia														
Slovenia[1]	D,G	x				x	x	x	x	x	x	x	x	
Spain[1]	D	x	x			x		x	x			x	x	
Sweden														
UK														
Non EU														
Iceland[1]	D	x	x	x	x	x	x	x	x		x			
Liechtenstein														
Norway[1]	D	x				x		x		x	x	x	x	

** France also tests for cefazolin (1st gen)*
†Luxembourg also tests for cefepime (4th gen)
‡Nalidixic acid/ciprofloxacin/ofloxacin
§ Sulfameracine/sulphonamide/trimethroprim/(sulfonamide)
*** Azithromycin*
††Amoxicillin/ampicillin
 # Ticarcillin/mezlocillin/piperacilin/mezlocillin&Sulbactam/ampicillin&sulbactam
§§ Amikacin/kanamycin/streptomycin/

[1] CLSI
[2] EUCAST
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)
[4] Deutsche Industrie Norm (DIN)
[5] Norwegian Working Group on Antimicrobials (NWGA)

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

Quality control

Ten out of the 21 laboratories participate in regular EQA programmes and/or inter-laboratory comparisons.

Most laboratories have internal quality control related to one or more of the services provided, however five laboratories indicate that they have no internal quality control and two provide no information. Eleven laboratories indicate that they participate in external quality assessment testing programmes for some or all of their activities, in particular related to isolation/identification (11) and species determination (11).

Eight laboratories indicate that they have accreditation for some or all of their services. Six laboratories are accredited to ISO 17025 and four to ISO 15189. One laboratory indicates that it is accredited to both standards.

Training

In many laboratories, training is regularly offered for bio-safety (13), quality control (12), identification/confirmation (11) and typing (11). Fewer laboratories offer training in AST (9) and accreditation (8). However, seven laboratories indicate that they do not provide staff training in any area.

Ten countries (11) provide training and/or guidelines on diagnostic procedures to primary laboratories, mainly in relation to identification and confirmation methods but also for typing, AST, bio-safety and quality control.

In relation to training needs, there was no specific area that ranked higher than the others.

Harmonisation

More than half of the laboratories follow national or international recommendations/guidelines for some or all of their activities including AST (12), further characterisation (13), confirmation (12) and isolation (9).

For epidemiological surveillance of *Yersinia* at EU-level PFGE was ranked highest as the most suitable typing method, followed by MLVA.

Harmonisation is required, particularly for serotyping and species determination. Bio-typing is suggested by three countries to be most important when harmonising methods.

7 *Listeria monocytogenes*

Responses were obtained from laboratories in 23 EU and two EEA countries. No response was obtained from Latvia, Portugal, Slovakia, Sweden or Liechtenstein. Some laboratories did not respond to all questions which is why the total number of responses is not 25 for all questions.

Reference laboratory services

Eleven of the 25 laboratories indicate that they receive all isolates (Austria, Belgium, Denmark, Finland, France, Iceland, Lithuania, Luxembourg, Norway, Romania and UK). Most of the remaining laboratories only receive isolates related to outbreak investigations and/or for diagnostic reasons. Six laboratories do not receive any outbreak-related isolates and Malta and Cyprus do not receive any isolates from other laboratories. The laboratory in Slovenia received a defined proportion of isolates.

In addition to the human isolates, 15 laboratories also process food isolates and nine laboratories process animal isolates.

In relation to direct sample/isolate processing services respondents provide further typing (20), antimicrobial susceptibility testing (9), primary isolation (13) and non-culture based identification (12). Most of the participating laboratories maintain a strain collection (21), with the exception of the laboratories in Cyprus, Lithuania and Malta.

In 12 laboratories all isolates are stored after examination, while eight other laboratories only store isolates in connection with an outbreak or when specifically asked to do so. In Malta no isolates are kept and in Romania a random sample of strains is stored. Cyprus and Greece did not provide information. For *Listeria monocytogenes* some laboratories are involved in a variety of other activities including method development (14), provision of reference material (14), isolation (13), identification and confirmation (12), training (11) and AST(9). Eight laboratories are involved in the provision of guidelines and the development of proposals for standardisation.

Nine laboratories (Bulgaria, Czech Republic, Denmark, Finland, Luxembourg, Netherlands, Norway, Poland and Slovenia) are situated within their national institutes of public health and eight others indicated that they have a working relationship with their institute. Estonia did not indicate a working relationship with the national institute of public health. Six laboratories did not provide information on this point. Collaboration generally covers support during outbreak investigations, typing methods, surveillance and research.

The number of isolates examined ranges from 0–1 525. In 2008, the NRL in France processed the highest number of isolates (1 525 strains of which 320 were clinical isolates). The UK laboratory analysed 1 362 isolates. The laboratory in Denmark processed about 180 isolates per year, Romania 168 and Spain 120. All other laboratories receive less than 100 isolates per year.

In 2008, the number of human isolates examined was less than or just above 50 at 11 laboratories. Six laboratories did not examine any human isolates (although in some cases this is likely to be because human isolates were examined in another laboratory). In 2008, the NRLs in France, Germany and the UK received 320, 180 and 169 isolates of human origin respectively.

All but three laboratories perform further typing of *Listeria monocytogenes*. The laboratory in Malta does not perform any further characterisation and Cyprus and Greece did not provide information on this topic.

Methods

Of the 23 laboratories, 20 use culture methods for the identification and confirmation of *L.monocytogenes*. In addition, seven laboratories also perform PCR, while two only perform PCR and do not use culture-based methods (Spain and UK).

Further characterisation and typing is performed in 22 laboratories and 20 of them do so on a routine basis. In six laboratories (France, Denmark, Lithuania, Luxembourg, Slovenia and UK) all the isolates are typed but in most laboratories typing is performed only on isolates from an outbreak or at the specific request of the laboratory sending the sample.

Of the laboratories that perform further characterisation, 17 use phenotypic methods. The phenotypic methods used for typing include serotyping by slide agglutination (17) and haemolysin production (16).

Molecular methods for further characterisation are used by 12 laboratories routinely and five occasionally. Molecular methods include PCR based 'serotyping' in five laboratories and PFGE in 13 (Table 8). MLVA is routinely performed in laboratories in Denmark and Norway and occasionally in Finland and France. AFLP is routinely used in the UK laboratory, as is virulence gene detection. Virulence gene detection is also occasionally used in the Czech Republic, Finland and France.

France, which has the WHO Collaboration Centre for *Listeria,* has developed an MLST scheme. This scheme was further developed or considered for development at laboratories in Belgium, Denmark, Ireland, Netherland, Poland and Slovenia.

The laboratories in Bulgaria and Lithuania hope to implement PFGE and the laboratory in the UK indicated plans to develop and implement a 'next-generation' typing strategy, including a combination of phylogenetic and virulence markers.

For epidemiological surveillance of *Listeria monocytogenes* at EU level, PFGE ranked highest and was considered the most suitable typing method.

Table 8: Overview of molecular typing methods for *Listeria monocytogenes* in 2012

	PFGE	MLVA
	N=13	N=4
EU		
Austria	x	
Belgium	x	
Bulgaria		
Cyprus		
Czech Republic	x	
Denmark	x	x
Estonia		
Finland		x
France	x	x
Germany		
Greece	x	
Hungary	x	
Ireland		
Italy	x	
Latvia		
Lithuania		
Luxembourg	x	
Malta		
Netherlands	x	
Poland	x	
Portugal		
Romania		
Slovakia		
Slovenia	x	
Spain		
Sweden		
United Kingdom		
Non EU		
Iceland		
Liechtenstein		
Norway	x	x

Antimicrobial susceptibility testing

AST is performed in eleven laboratories: Austria, Belgium, Bulgaria, Czech Republic, France, Hungary, Iceland, Lithuania, Luxembourg, Romania and the UK. In the laboratories in Austria, Belgium, Bulgaria, France, Iceland, Luxembourg and Lithuania AST is routinely performed on all isolates, while in Hungary AST is only performed during outbreaks or at the request of the laboratory sending the sample. At the laboratories in the Czech Republic, Romania and the United Kingdom, AST is usually performed in accordance with a specific request.

AST is performed by means of disc diffusion in seven laboratories. The gradient strip MIC method (6) and dilution susceptibility (1) are also used. A number of laboratories use more than one method to perform AST.

The number of antimicrobials tested range between five and 22. All laboratories report AST testing for penicillins and erythromycin (Table 9). Other antibiotics regularly included were tetracycline (9), trimethoprim (8), choramphenicol (6) and ciprofloxacin (7). Some laboratories indicate that they test susceptibility to cefotaxime (although *L. monocytogenes* is intrinsically resistant to this agent).

For interpretation of AST, most laboratories indicate that they use CLSI (9) and/or EUCAST (7). The NRL in France uses the French Society for Microbiology Guidelines and this is the only laboratory that identifies resistance genes in *L.monocytogenes* using molecular techniques. Some laboratories report using more than one method for measurement and/or interpretation of susceptibility test results.

Belgium, France and Iceland have national surveillance programmes for *L.monocytogenes* antimicrobial resistance.

Table 9: Overview of methods and antimicrobial agents tested for Listeria *monocytogenes* in 2012

CLASS		Penicillins		Carbapenems	Quinolones*	Aminoglycosides		Sulfonamides	Chloramphenicol	Tetracyclines	Macrolides†
Antimicrobial agent		Aminopenicillins‡	Other§	Imipenem**		Gentamicin	Other††				Erythromycin
	Method	N=10	N=4	N=6	N=7	N=9	N=6	N=8	N=6	N=9	N=11
EU											
Austria[1,2]	D,G	x		x	x	x		x	x	x	x
Belgium[1]	G	x			x	x	x	x	x	x	x
Bulgaria[1,2]	D			x		x				x	x
Cyprus											
Czech Republic[1,2]	D,G	x	x		x	x	x	x	x	x	x
Denmark											
Estonia[2]	D	x		x				x			x
Finland											
France[1,2,3]	D,G	x		x	x	x	x	x	x	x	x
Germany											
Greece											
Hungary[1]	G	x	x			x	x	x	x	x	x
Ireland											
Italy											
Latvia											
Lithuania											
Luxembourg[1,2]	D	x	x	x	x	x	x	x		x	x
Malta											
Netherlands											
Poland											
Portugal											
Romania[1]	D	x		x	x	x			x	x	x
Slovakia											
Slovenia											
Spain											
Sweden											
UK[2]	C	x			x		x			x	x
Non EU											
Iceland[1]	G	x	x			x		x			x
Liechtenstein											
Norway											

**Nalidixic acid/ciprofloxacin/levofloxacin*
† Erythromycin/azithromycin

‡Amoxicillin/ampicillin
§ Mezlocillin/oxacillin/penicillin

*** Meropenem instead of imipenem in Estonia*
†† Amikacin/kanamycin/streptomycin/tobramycin

[1] CLSI
[2] EUCAST (note EUCAST provide clinical breakpoint interpretive criteria only for ampicillin, benzylpenicillin,erythromycin, meropenem and trimethoprim-sulfamethoxaxole)
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

Quality control

Five out of 23 laboratories report participation in regular EQA programmes.

Internal quality control programmes are in place at 17 laboratories, mainly for identification and confirmation purposes (17) but also for serotyping (14) and AST (6). Four laboratories indicate that they have no internal quality control in any area specified and two do not provide information on this issue.

Seven laboratories participate in external quality assessment programmes for identification and confirmation in relation to some or their activities, including identification, AST and serotyping.

Eight laboratories indicate that they are accredited for some or all of their services. Most of these laboratories are accredited to the ISO 17025 standard (6), although some are accredited to the ISO 15189 standard instead or in addition to ISO 17025. Two laboratories were in the process of implementing or obtaining accreditation to ISO 15189 at the time of the survey.

Training

Training is regularly offered for bio-safety, quality control and typing in many laboratories, however seven laboratories indicated that no training was provided in any of the areas specified. One laboratory did not provide any information on training.

In terms of training needs, training in typing methods was ranked slightly higher than other areas which generally received a similar ranking.

Harmonisation

Of the 20 respondents to this part of the questionnaire, 16 indicated that they follow national or international guideline recommendations for some or all of their activities. Few laboratories offered reference services to other countries, with the exception of France, where the NRL for *Listeria* and the WHO Collaborating Centre are located. The NRL in France offers all services, from isolation, confirmation, serotyping and AST to genotyping. The laboratories in the Czech Republic and Hungary also provide some services to other countries. Belgium and Hungary sometimes request services from other reference laboratories for *Listeria*, usually for species determination, serotyping or phage typing.

For harmonisation purposes, (geno)serotyping was ranked highest, followed by virulence gene detection and AST. Molecular typing methods, MLVA and PFGE, need to be standardised, as specifically stated by nine laboratories.

8 STEC/VTEC

Responses were obtained from laboratories in 23 EU countries and two EEA countries. No response was obtained from Latvia, Portugal, Slovakia, the United Kingdom or Liechtenstein. Some laboratories did not respond to all questions which is why the total number of responses is not 25 for all questions.

Reference laboratory services

A majority (14) of the laboratories received all isolates (Belgium, Czech Republic, Denmark, Finland, Greece, Hungary, Iceland, Ireland, Lithuania, Luxembourg, Norway, Poland, Slovenia, and Sweden). In nine other countries isolates were only sent for diagnostic purposes and/or outbreak investigation. In addition, laboratories in Germany, the Netherlands, Romania and Spain received additional isolates, although no further information was provided on the basis for isolate submission.

Of the 23 laboratories that responded to the question, 15 processed STEC/VTEC isolates from food samples and 12 from animal samples.

In relation to direct sample processing most respondents provided primary isolation (20), non-culture based identification (15), further typing (23) and AST (16). Nearly all laboratories (20) maintain a strain collection with the exception of Malta, Estonia and Lithuania.

Most laboratories store the STEC/VTEC strains routinely (22) or occasionally (2). Of these 17 store all isolates and six only store strains in connection with an outbreak or if specifically requested.

Almost all (23) laboratories indicated that they were involved in further typing and provision of microbiological advice. Most other laboratories also provide support during outbreaks (22). Laboratories participate in research and scientific publications (18), AST (16) and method development (17) to a lesser extent. Other activities were provided less often, including training (14), support in quality assurance (13), standardisation of methods (12), provision of reference materials (12), provision of guidelines (11), organisation of trials (6) and provision of material for proficiency testing (6).

There are nine laboratories located within national institutes for public health in their respective countries and another 11 have a working relationship with their national institutes. This collaboration mainly relates to surveillance and outbreak investigations, typing methods and research. The Czech Republic, Bulgaria and Greece did not indicate any collaboration with their national institutes.

The number of human STEC/VTEC isolates analysed was highest at laboratories in the Netherlands 1 133. In Germany there were around 350, more than 200 in Sweden and Ireland and around 150 in Denmark and Hungary. Belgium Bulgaria, France and Lithuania received 100 strains or less and around 50 or less were received in Austria, the Czech Republic, Cyprus, Estonia, Finland, Iceland, Italy, Luxembourg, Malta, Poland, Norway, Romania, Slovenia and Spain.

Methods

Identification and confirmation of STEC/VTEC is usually performed using culture methods (24), serotyping (20) and PCR (19).

For detection of STEC/VTEC, the selective culture media most often mentioned is CT-SMAC (Mac Conkey Sorbitol Agar) (16). Other less frequently mentioned media are SMAC (9), Enterohemolysin agar (5) and EHEC direct medium (2).

Further characterisation of the STEC/VTEC is performed by all 25 laboratories. Further characterisation is generally performed during outbreak investigations (19) and when specifically requested by the laboratory sending the sample (15). All isolates submitted are further typed in Cyprus, Denmark, Finland, Iceland, Ireland, Lithuania, Luxembourg, Norway, Slovenia, Sweden and Spain.

All 25 laboratories use some phenotypic methods for further characterisation. Phenotypic methods for characterisation often mentioned are sorbitol fermentation (24), beta glucuronidase production (16) or haemolysin production (15). Phage typing is less widely used (5). Verotoxin testing is performed in 17 laboratories (15 routinely) by EIA (Enzyme Immuno Assay) (9) or Vero cell assay.

Molecular methods are widely used, including toxin gene detection (18). Molecular typing is performed in 20 countries. Most often this entails PFGE (20). Other methods used are MLVA (6), MLST (France and Spain) and RLFP (Belgium, Czech Republic, France and Poland) (Table 10).

O-grouping is performed in all countries, and O157 is always assessed. In five countries, the full range (O1–O181) is performed. Malta and Greece only include O157 in the O-grouping. Finland, Lithuania, Luxembourg, Poland,

Romania and Spain include at least O157, O111 and O26. Austria, Belgium, the Czech Republic, Ireland, Italy and Norway include at least O157, O111, O26, O103 and O145.

Phenotypic O-grouping is always done by slide agglutination and additionally by tube agglutination in some laboratories. Genotypic O-grouping is carried out by 13 countries.

H-typing is done in 15 laboratories, most of which perform phenotypic H-typing (14) and genotypic H-typing is offered to some degree by 10 laboratories (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands, Norway, Spain and Sweden). Denmark, Estonia, Germany, Finland, the Netherlands and Sweden include the whole range of H antigens (H1-H53) for assessment. The other laboratories always test for H7.

Virulence gene detection is performed in 21 laboratories. The virulence genes generally tested for are vtx1 (21), vtx2 (21), intimin gene (20) and haemolysin A gene (19). Subsequent subtyping is performed on the vtx2 gene by 14 laboratories, and on both the vtx1 and intimin gene by seven countries.

Of 13 laboratories planning to implement an additional typing method, six plan to use MLVA and another five laboratories plan (extended) vtx(s) and eae subtyping (Table 10). Typing via microarray (2) and MLST (3) are also mentioned.

When asked which molecular typing method would be most suitable for epidemiological surveillance purposes at EU level, PFGE is ranked highest, followed by MLVA. Other suggestions are vtx1 and vtx2 sequencing.

Table 10: Overview of molecular typing methods for STEC/VTEC in 2012

	PFGE	MLVA	MLST	Others
	N=20	N=6	N=2	N=6
EU				
Austria	x			
Belgium	x	x		x
Bulgaria		x		
Cyprus				
Czech Republic	x			x
Denmark	x			
Estonia				x
Finland	x			
France	x		x	x
Germany	x			
Greece	x			
Hungary	x			
Ireland	x			
Italy	x			
Latvia				
Lithuania				
Luxembourg	x	x		x
Malta				
Netherlands	x			
Poland	x			x
Portugal				
Romania	x			
Slovakia				
Slovenia	x			
Spain	x	x	x	
Sweden	x	x		
United Kingdom				
Non EU				
Iceland	x			
Liechtenstein				
Norway	x	x		

Antimicrobial susceptibility testing

In five countries there is a national surveillance programme in place for antimicrobial resistance in STEC/VTEC: (Belgium, Denmark, Iceland, Ireland and Slovenia).

Most laboratories (17) perform AST on all isolates received during outbreaks or if otherwise specifically requested.

Disc diffusion is most often used (13) but gradient strip MIC methods are also applied in six laboratories, as are dilution methods in two. A number of laboratories use more than one method.

When performing AST, a panel of a minimum of seven (Hungary and Italy) and maximum 19 (Denmark) antimicrobial agents is tested (Table 11), with an average of 13. Ciprofloxacin is always part of the panel (17). An aminopenicillin (ampicillin in 15 and amoxicillin in France) is included in 16 laboratories and all test for susceptibility to cefotaxime or ceftriaxone (some capacity to detect ESBLs). Trimethoprim either alone or in combination with sulphamethoxazole (co-trimoxazole) is included by 15 laboratories. Few laboratories test for susceptibility to any carbapenem (six test for detection of CPE) or to a macrolide (2). Other commonly used antimicrobials are streptomycin by 10 and sulphonamides by nine laboratories.

The guidelines applied to determine interpretive breakpoints are generally CLSI (15) and EUCAST for two countries. National guidelines are also applied by some countries. Some laboratories indicate that they use more than one set of methods or interpretive criteria.

Some laboratories (Denmark, Hungary, Ireland, Italy, Romania and Spain) also identify antibiotic resistance genes in STEC/VTEC using molecular techniques.

Table 11: Overview of methods and antimicrobial agents tested for STEC/VTEC in 2012

CLASS		Penicillins			Cephalosporins*†		Carbapenems	Quinolones‡	Aminoglycosides		Sulfonamides§	Chloramphenicol	Tetracyclines	Macrolides**
Antimicrobial agent		Aminopenicillins††	Amoxicillin-clavulanic acid	Other	Second	Third	Imipenem		Gentamicin	Other#				Erythromycin
	Method	N=16	N=11	N=7	N=6	N=17	N=6	N=17	N=16	N=12	N=15	N=14	N=15	N=2
EU														
Austria														
Belgium[1]	D	x	x			x		x	x	x	x	x	x	
Bulgaria[1]	D	x	x		x	x		x	x	x		x	x	
Cyprus[1]	C	x	x	x	x	x	x	x	x		x		x	
Czech Republic														
Denmark[2]	C	x	x	x		x		x	x	x	x	x	x	
Estonia														
Finland[1,2]	D,G	x		x		x	x	x	x	x	x	x	x	
France[3]	D,G	x				x		x	x	x	x	x	x	
Germany[1,4]	C	x		x	x	x		x	x	x	x	x	x	x
Greece														
Hungary[1]	D	x				x		x			x	x	x	
Ireland[1]	D	x				x		x	x	x	x	x	x	
Italy[1]	D					x		x	x			x	x	
Latvia														
Lithuania														
Luxembourg[1]	D	x	x	x		x		x	x	x	x	x	x	
Malta[1]	G	x	x	x	x	x	x	x	x	x	x			
Netherlands														
Poland[1]	D	x	x			x		x	x	x	x	x	x	
Portugal														
Romania[1]	D,G	x	x		x	x	x	x	x	x	x	x	x	
Slovakia														
Slovenia[1]	D,G	x	x			x	x	x	x		x	x	x	
Spain[1]	D,G	x	x			x		x	x	x	x	x	x	
Sweden														
UK														
Non EU														
Iceland[1]	D	x	x	x	x	x	x	x	x		x			x
Liechtenstein														
Norway														

** Luxembourg also tested for cefepime*
† Malta also tested for cefazolin
‡ Nalidixic acid/ciprofloxacin
§ Sulfameracine/sulphonamide/trimethoprim(sulfonamide
*** Azithromycin*
†† Amoxicillin/ampicillin
 # Amikacin/apramycin/kanamycin/neomycin/streptomycin

[1] CLSI
[2] EUCAST
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)
[4] Deutsche Industrie Norm (DIN)

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

Quality control

The majority of laboratories (21) participate in regular EQA programmes or inter-laboratory comparison. Internal quality control programmes exist at most laboratories for some or all activities including identification and confirmation (15), typing (14) and AST (12). Six laboratories have no internal quality control in any of their areas of activity and one laboratory provided no information on internal quality control.

Nineteen (19) laboratories participate in external quality assessment programmes for identification and confirmation, mainly the ECDC scheme (9), but also UK NEQAS (6), WHO GFN (2) and INSTAND (1). A total of 22 countries have external quality assessment for typing, mainly provided by ECDC (18).

Fourteen laboratories indicated that they are accredited for some or all of the VTEC/STEC services provided. At 11 laboratories the accreditation was to ISO 17025 standard at four to ISO 15189. Another three laboratories are applying for accreditation.

Training

Most laboratories have staff training for some or all of their activities but nine laboratories indicated that they did not have staff training activities in any of the areas specified and one provided no information on this issue.

Amongst those areas in which training is provided, 16 of the 25 laboratories have regular bio-safety training, and 15 of these 25 receive training in identification and confirmation methods, and 14 of 25 in typing, AST and quality control/assurance. The laboratories gave a higher ranking to training needs in relation to typing methods, quality control issues and AST methods.

Harmonisation

Harmonisation of methods shows that when processing STEC/VTEC, national or international guidance or recommendations are followed for many activities including AST (14) and for further characterisation/typing and isolation and confirmation.

Serotyping, virulence detection, and verotoxin gene detection were identified as those activities most in need of harmonisation at the EU level. Some laboratories that are considering implementing additional methods indicated that harmonisation would help them to make a decision as to which new method would be best.

9 *Shigella*

Questionnaires were returned from the laboratories in 23 EU and two EEA countries. The laboratory from the Netherlands did not complete a questionnaire but indicated that, although there is no NRL for *Shigella* some services are provided. No responses were obtained from Latvia, Portugal, the United Kingdom or Liechtenstein. Some laboratories did not respond to all questions which is why the total number of responses is not 25 for all questions.

Reference laboratory services

Twenty one (21) respondents indicated that all *Shigella* isolates are sent to the NRL. In Ireland, all isolates are requested but not all are submitted and in Lithuania isolates are submitted only if they have not been identified by the primary laboratory. In Malta, the laboratory that responded to the questionnaire does not accept any isolates from other laboratories.

In relation to direct sample/isolate processing, most respondents indicated that they provide further typing (23, not in Poland and Czech Republic), antimicrobial susceptibility testing (22, not in Slovakia, Czech Republic and Sweden), primary isolation (14) and non-culture based identification (13). Most laboratories (22) maintain a strain collection with the exception of Estonia, Lithuania and Malta.

All laboratories use culture methods for identification and confirmation of *Shigella*, while 12 also use non-culture based methods. In 17 laboratories all of the isolates submitted are stored but none are stored in Lithuania and Malta. The laboratories in six countries (Estonia, Hungary, Poland, Romania, Slovakia and Spain) only store isolates from outbreaks or when specifically requested.

All laboratories are involved in the provision of microbiological advice (25) and support in outbreak investigation (25). Other support services offered by most laboratories are research training (17), method development (15) and research, and scientific publication (17). To a lesser extent, laboratories are involved in support for quality assurance in primary laboratories (13), guidelines on diagnostic procedures for primary laboratories in their own country (12), providing material for proficiency testing organisers (11), providing reference material to primary laboratories (11) and organising ring trials (5).

Nine laboratories (Bulgaria, Denmark, Finland, Hungary, Italy, Luxembourg, Norway, Slovenia and Sweden) are part of the national institute for public health in their country, while 13 other NRLs work closely with their national institute. This working relationship covers surveillance, outbreak investigations, typing methods and, to a lesser extent, research. Two laboratories do not have a national institute for public health and one did not answer the question.

The total number of isolates confirmed or further characterised varied between zero (Cyprus) and 844 (France), with an average of 134 (median 40). Only France and Sweden have more than 500 isolates, Bulgaria and Belgium have just under 500, Austria, Denmark, Norway and Finland analysed just over 100 strains, Spain 70 and the remaining 16 countries analysed less than 50 strains in 2008.

Methods

All laboratories use culture methods for identification and confirmation of *Shigella*, while 12 also use non-culture based methods.

Routinely used selective media were XLD (7), *Salmonella-Shigella* agar (4) and Hektoen (4). Seven countries did not describe the media used.

Further characterisation and typing of *Shigella* is done routinely by 24 laboratories and occasionally in Spain.

Phenotypic methods for further typing are used in all laboratories, with all using serological methods and just four using phage typing. Molecular methods are used by 17 laboratories including PFGE (17), virulence gene detection (7), plasmid profile analysis (5) and MLST (2) and MLVA (Norway) (Table 12). In nine laboratories, all isolates are further characterised while in others this is limited to specific requests (15) and/or outbreak investigations (15).

Respondents generally considered PFGE as the most suitable method for epidemiological surveillance of *Shigella* at EU level, with AFLP as another potential option. Eight laboratories plan to implement other typing methods including PFGE in a further three laboratories, MLST in three (all are already providing PFGE) and MLVA in two (neither offering PFGE).

Table 12: Overview of molecular typing methods for *Shigella* spp. in 2012

	PFGE	MLST	Plasmid profiling
	N=17	N=2	N=5
EU			
Austria	x		
Belgium	x		x
Bulgaria			
Cyprus			
Czech Republic			
Denmark	x		
Estonia			
Finland	x		
France	x	x	
Germany	x		x
Greece	x		
Hungary	x		x
Ireland	x		x
Italy	x		
Latvia			
Lithuania			
Luxembourg	x		
Malta			
Netherlands			
Poland	x		
Portugal			
Romania	x		
Slovakia			
Slovenia	x		
Spain	x	x	x
Sweden	x		
United Kingdom			
Non EU			
Iceland			
Liechtenstein			
Norway	x		

Antimicrobial susceptibility testing

Six out of the 24 countries have a surveillance programme for antimicrobial resistance in *Shigella* (Austria, Belgium, Greece, Iceland, Norway and Poland). All but three laboratories (Czech Republic, Slovakia and Sweden) perform AST for *Shigella*. The most widely used method for AST is disc diffusion (18) and gradient-strip MIC testing is also occasionally used (9). Dilution susceptibility is used in only four laboratories.

For interpretation, the criteria most often used are CLSI (17), with three countries using EUCAST. Norway and France use their own national guidelines on breakpoints.

A number of laboratories indicated that they use more than one method for performing AST and more than one set of criteria for interpretation.

On average, susceptibility to 11 antimicrobials is tested, with a minimum of four (Cyprus and Estonia) and a maximum of 21 (Austria) (Table 13). Ciprofloxacin is always part of the panel. An aminopenicillin (ampicillin in 21 and amoxicillin in France) is included in all laboratories and all but three laboratories (Estonia, Luxembourg and Norway) are testing for susceptibility to cefotaxime or ceftriaxone and therefore have some capacity to detect ESBLs. Five countries (Austria, Finland, Malta, Romania and Slovenia) test for imipenem and so may be able to detect carbapemenase producers (CPE). Panels also frequently include chloramphenicol (20 except Cyprus), nalidixic acid (18), tetracycline (18), gentamicin (16) and trimethoprim (19, ten of which are in combination with sulphamethoxazole).

Table 13: Overview of methods and antimicrobial agents tested for *Shigella* spp. in 2012

CLASS		Penicillins			Cephalosporins*		Carbapenems	Quinolones†	Nalidixic acid	Aminoglycosides		Sulfonamides‡	Chloramphenicol	Tetracyclines	Macrolides
Antimicrobial agent		Aminopenicillins§	Amoxicillin-clavulanic acid	Other**	Second	Third	Imipenem			Gentamicin	Other††				Erythromycin
	Method	N=22	N=12	N=5	N=3	N=21	N=5	N=22	N=18	N=16	N=15	N=19	N=20	N=18	N=8
EU															
Austria[1]	D,G	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Belgium[1]	D	x	x			x		x	x	x	x	x	x	x	x
Bulgaria[1]	C,D,G	x	x		x	x		x	x	x	x	x	x	x	
Cyprus[1]	C	x				x		x			x	x			
Czech Republic															
Denmark[2]	C	x	x			x		x	x	x	x	x	x	x	x
Estonia[1,2]	D	x				x		x				x			
Finland[1,2]	D,G	x		x		x	x	x	x	x	x	x	x	x	
France[3]	D,G	x	x			x		x	x	x	x	x	x	x	x
Germany[4]	C	x		x	x	x		x	x	x	x	x	x	x	x
Greece[1]	D	x	x			x		x	x	x	x	x	x	x	x
Hungary[1]	D	x				x		x	x			x	x	x	
Ireland[1]	D,G	x				x		x	x	x	x	x	x	x	
Italy[1]	D,G	x				x		x	x	x			x	x	x
Latvia															
Lithuania[1]	D	x						x		x			x	x	
Luxembourg[1]	D,G	x	x	x		x		x	x	x	x	x	x	x	x
Malta[1]	G	x	x	x		x	x	x		x	x		x		
Netherlands															
Poland[1]	D	x	x			x		x	x	x	x	x	x	x	
Portugal															
Romania[1]	D	x	x			x	x	x	x		x	x	x	x	
Slovakia															
Slovenia[1]	D,G	x	x			x	x	x	x	x		x	x	x	
Spain[1]	D	x	x			x		x	x	x	x	x	x	x	
Sweden															
UK															
Non EU															
Iceland[1]	D	x				x		x	x			x	x		
Liechtenstein															
Norway[5]	D	x				x		x	x			x	x	x	

** Luxembourg also tests for cefepime*
† Nalidixic acid/ciprofloxacin/ofloxacin
‡ Sulfameracine/sulfonamides/sulphamethoxazole/trimethroprim/trimethroprim-sulphamethoxazole/Cotrimoxazole
§ Amoxicillin/ampicillin
*** Mecillinam, mezlocillin/piperacilin*
†† Amikacin/apramycin/kanamycin/neomycin/netilmicin

[1] CLSI
[2] EUCAST
[3] Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM)
[4] Deutsche Industrie Norm (DIN)
[5] Norwegian Working Group on Antimicrobials (NWGA)

C = Dilution (Minimum Inhibitory Concentration)
D = Disk diffusion
G = Gradient strip MIC method

Quality control

Nearly half of the laboratories participate in EQA programmes or inter-laboratory comparison schemes (10). AST is commonly addressed via internal quality control schemes (16) but only four laboratories report participation in an external quality assessment scheme (Belgium, Cyprus, Czech Republic and Ireland). For identification, seven laboratories participate in UK NEQAS and a further seven laboratories in the WHO GFN EQA scheme. For species determination, the same seven laboratories participate in UK NEQAS and three in WHO GFN.

Nine of the 22 laboratories report that they are accredited for identification, species determination and serotyping, only one for phage typing (Austria) and six for AST. Austria, Belgium, Bulgaria, Czech Republic, Denmark, Hungary and Poland have accreditation according to the ISO 17025 standard, and the Czech Republic, Estonia and Hungary are accredited to the ISO 15189 standard. Other laboratories are planning to apply for accreditation (ISO 15189): Greece, Iceland, Ireland and Slovenia.

Training

Staff training is offered regularly in 18 laboratories for some or all of their activities including identification (16), typing (15), AST (13), bio-safety (18) and quality control (16). The respondents indicate less clarity on priorities for training in relation to *Shigella* than *Campylobacter* or *Salmonella*. Ranking is generally around three, indicating that it was not possible to identify any real difference in priority of training needs.

Twelve countries provide training and/or guidelines on diagnostic procedures to primary laboratories, mainly in relation to identification and confirmation methods (13) but also for typing (11), quality control (6), AST (5) and bio-safety (5). The other twelve do not provide any training or guidelines.

Harmonisation

Of the 22 laboratories providing responses, the majority indicate that international recommendations/guidance for AST are followed (17).

For epidemiological surveillance of *Shigella* at EU level, PFGE was given the highest ranking as the most suitable typing method.

Harmonisation is indicated and ranked as deserving some priority for virulence gene detection and phenotyping.

10 Discussion

Surveillance of infectious disease supports identification of priorities for action and the planning of effective intervention. Comprehensive surveillance of infectious disease requires clinical and laboratory-based surveillance. Clinical surveillance systems can provide essential and timely information on the occurrence of specific clinical syndromes and their temporal and spatial distribution (for example gastroenteritis). However, clinical surveillance has limitations in that it cannot be specific as to the pathogen and in many countries, even where a disease is notifiable, clinical reporting may be the exception rather than the rule. The importance of microbiology referral laboratories for protection of public health is reflected in the 2010 ECDC report *Core functions of microbiology reference laboratories for communicable diseases* , the *Update on the position statement of the Commission and ECDC on human pathogen laboratories* (ECDC Management Board, November 2011) and the International Health Regulations 2005.

In the context of growing European integration, with free movement of people and a single market for food, being able to manage outbreaks of food- and waterborne infection in an international context is increasingly important. A network of quality-assured referral laboratories in different Member States is critical to Europe's capacity for early detection and response to outbreaks of food- and waterborne diseases, as well as offering the best available surveillance system for sporadic disease. Standardisation/harmonisation of identification and characterisation of priority pathogens is a vital element in the communication and exchange of information.

Food- and waterborne diseases pose specific challenges. Many food- and waterborne infections manifest clinically as gastroenteritis/infectious intestinal disease which is so common that it often goes unreported. Gastrointestinal diseases are generally not attributable to a particular pathogen in the absence of laboratory diagnosis. However, the burden of illness is considerable. A recent UK study indicated that there were 274 cases of infectious intestinal disease per 1 000 of population per year, with 147 community cases and ten doctor's consultations for each case documented by UK national surveillance.

Accreditation of medical laboratories is a process for assuring quality of medical laboratory services. Although a system of European co-operation for Accreditation (EA) is in place, accreditation of medical laboratories is not mandated in EU legislation. Another important element to supporting quality in medical microbiology laboratories is the existence of expert NRLs. In *Core functions of microbiology reference laboratories for communicable diseases* the first core function, reference diagnostics, relates to capacity to accept microorganisms isolated (isolates) from primary medical laboratories to confirm their findings. NRLs also support primary clinical laboratories by providing reference materials, scientific advice and collaboration and research. NRLs are therefore not only a source of laboratory-based surveillance data, but also a means for assuring the quality and developing the services of state/regional/local laboratories.

An important part of the 'reference diagnostics' function of NRLs is to perform further detailed characterisation of the isolates submitted. Referral laboratories can confirm suspected outbreaks of infection and identify unsuspected outbreaks through application of an increasingly sophisticated range of analytical methods and data analysis, such as PFGE and MLVA. Working with relevant public health agencies NRLs play an important role in supporting measures to control infection, particularly if the analytical methods applied to isolates from human infection are integrated with data from food, water and animals. This emphasises the need to include veterinary reference laboratories in the discussions on method standardisation and harmonisation to ensure comparability of applied analytical testing methods and reported data.

This report presents the most comprehensive picture to date of the of the extent to which NRL services are available in the Member States to deal with the six priority FWD pathogens: *Campylobacter* spp., L. *monocytogenes*, *Salmonella* spp., *Shigella* spp, verocytotoxin-producing *E. coli* (VTEC) and *Yersinia* spp. The report outlines the range of services provided, the extent to which quality assurance systems are in place and the plans for development and training needs of the referral laboratories.

Participation in the survey was generally excellent (overall response rate of >80%). A response was obtained from a laboratory in most Member States for most pathogens. However, some NRLs did not respond while in other countries detailed responses were included from laboratories with significant relevant expertise but not designated referral laboratories. The broad picture that emerges is one of considerable heterogeneity. There is little consistency in terms of overall NRL capacity of individual Member States and within Member States there is significant inconsistency with respect to the six individual pathogens.

Some Member States have a comprehensive quality-assured NRL network for the full range of pathogens assessed while other Member States have no formal referral laboratory services for any one of the six pathogens examined.

In most countries the work of the NRLs is integrated into the national institute for public health, but some countries report no relationship with their national institute for public health which hampers integrated action, in particular during outbreaks.

There is no consistency in the extent to which laboratories accept isolates from food and animals. Integrating data from clinical isolates of a food- or waterborne disease with data from food and animals is important for identification of its source. However, this process does not require the isolates to be processed at a single laboratory, provided the methodology is comparable and the data are integrated. Information on the existence of systems for the integration of data from human and non human isolates was, however, beyond the scope of this survey.

Campylobacter is the most common bacterial cause of gastrointestinal infection in Europe. Yet not all countries have a dedicated NRL, while some have designated laboratories but examine only a handful of isolates. Other countries have NRLs where sophisticated methods are applied to a substantial number of isolates. The difference in relation to capacity may be connected to the relatively late recognition of *Campylobacter* as an important FWD pathogen and the failure to date to routinely apply phenotypic typing methods to identify significant numbers of discrete outbreaks. The increasing application of molecular methods has made important contributions to the understanding of the general epidemiology of *Campylobacter* infection. The application of novel, sequence-based methods may greatly enhance the process of linking cases with one another and with specific sources.

Responses were most complete and referral services most comprehensive for *Salmonella*. In almost all cases laboratories describe services for serotyping and AST as a minimum. This may reflect the longstanding recognition of the significance of this pathogen and the long-established phenotypic systems (serotyping) for discriminating between different serovars. *Salmonella* also has the propensity to be associated with large identifiable point source outbreaks, although the number of outbreaks appears to be declining. Many laboratories also perform phage typing and relatively comprehensive AST, however there is considerable diversity in the number and kind of antimicrobial agents tested. Many of the laboratories also have one or more molecular typing methods for *Salmonella* (predominantly PFGE and MLVA).

For *Yersinia* spp., there is considerable heterogeneity between NRLs and this could be due in part to major differences in the reported incidence of infection for *Yersina* spp. in different regions of Europe. Services are very well developed in some countries while others have no reference laboratory services and report very few isolates.

Unlike the other five priority pathogens considered here *Shigella* spp. is primarily transmitted directly or indirectly from person to person rather than from food or animals. Given the number of human cases reported, European NRL services are generally quite well developed, although here too there are striking differences between Member States' capacity.

L. monocytogenes is distinctive in that it accounts for a relatively small number of infections but with a high mortality, it has a long incubation period and exposure is unlikely to cause recognised disease in otherwise healthy non-pregnant people. As a consequence, timely laboratory detection and detailed characterisation of isolates may be particularly important in linking cases with one another and with food products. The capacity of some laboratories is extensive while others appear only to be able to deal with species level identification.

Wherever tests are performed to identify STEC/VTEC, it appears to be associated with disease. However, there is no consistency in terms of referral laboratory services. Some laboratories have very sophisticated methods for detection, identification and subtyping this complex and challenging group of pathogenic *E. coli*. The complexity and plasticity of this group of pathogens and the need for such methods was brought into sharp relief in 2011 with the Shiga toxin -producing enteroaggregative *E. coli* outbreak in Germany. Although, at the time this survey was conducted, a proportion of responding laboratories were able to confirm toxin genes and identify the five most common O serogroups, there were a number of countries that apparently had no provision for referral laboratory services for STEC/VTEC. Moreover there were some that indicated they only had the capacity to confirm the O157 antigen.

AST to guide immediate patient care is often not necessary in patients with gastroenteritis and when necessary it is typically performed at primary medical laboratories. However AST in referral laboratories is valuable for a number of reasons. As with other methods of characterisation, performing AST at the referral laboratory is useful for the laboratory itself in evaluating its own performance and identifying deficiencies. Patterns of antimicrobial resistance in a number of pathogens (notably *Salmonella* and *Shigella*) are commonly used as phenotypic markers of value in assessing possible relationships between isolates from different sources, e.g. the *S.* Typhimurium clonal group DT104 is characterised by a pattern of resistance to five antimicrobial agents. Data from referral laboratories also provides a basis for surveillance of new and emerging antimicrobial resistance patterns.

Most laboratories responding to this survey describe comprehensive AST testing using standardised methods and interpretive criteria. However, others report limited or no AST testing and considerable heterogeneity regarding the range of antimicrobial agents tested. Most laboratories include testing for agents relevant to detection of some emerging antimicrobial resistance threats. More specifically, they test for third generation cephalosporins, which should allow detection of most of the ESBL producers and for quinolone or fluoroquinolone agents. However, few laboratories test for carbapenems and therefore have limited capacity to detect carbapenemase producers (CPEs). CPEs are recognised as a major threat to public health in the EU. Carbapenemase production in *Salmonella* has been reported and laboratory evidence has shown the transfer of the resistance determinants to *Salmonella* Typhimurium.

Overall disc diffusion methods are most widely used for AST. Dilution methods and gradient strips methods are less widely used. Dilution methods for surveillance of AST are emphasised by EFSA but most referral laboratories do not appear to be able to provide corresponding data for human isolates. However, methods used to categorise isolates as susceptible and resistant (or wild-type/non wild-type) by disc diffusion and dilution are generally similar, provided the interpretive criteria applied to the zone of inhibition diameters are properly derived from the corresponding criteria for minimum inhibitory concentrations. At the time of the survey, the most widely used methods and interpretive criteria were those of the CLSI although some laboratories were using EUCAST. The promotion of EUCAST methods and criteria in recent years may have changed the situation since the survey was conducted.

In general, responses show a strong focus on quality in many laboratories. Most laboratories report comprehensive internal quality control or at least some elements of this. Most laboratories participate in ring trials/external quality assessment programmes where available. The importance of European (ECDC) and global (WHO) services in supporting the quality of referral laboratories is apparent as these are often the only specialised EQA available.

For most pathogens around half of the laboratories indicate that they have accreditation for some or all of their activities, according to the ISO 17025 or ISO 15189 standards. A number of those not accredited are working towards this goal. However, there is some inconsistency in responses as some laboratories state that they do not have internal quality control or staff training but have accreditation. Most laboratories report ongoing staff training related to some or all of their areas of activity although some have no staff training of any kind. External training needs in a number of areas specific to each pathogen have been identified. For *Campylobacter*, *Salmonella*, *Listeria* and VTEC training needs for typing were ranked highest, followed by AST and quality control. For *Yersinia* and *Shigella* no specific needs were ranked higher than any others.

Various laboratories have indicated plans to expand their scope of activities over the next few years. Some laboratories plan to expand their range of phenotypic tests but most plan to introduce or expand molecular typing methods. PFGE is often considered, for introduction or expansion, and more sophisticated laboratories intend to introduce or expand the application of MLVA and sequence-based typing methods including MLST. There is a general recognition of the need for greater harmonisation of methods among European referral laboratories. The particular areas of emphasis are pathogen-specific but there is a general emphasis on harmonisation of molecular methods and AST.

Considerable care was taken in developing this questionnaire to ensure that the data collected form a basis for the most comprehensive picture to date of referral laboratory services for food- and waterborne pathogens in Europe. It is apparent, however, that some questions were not understood in the same way by all respondents and the level of detail in the questionnaires may have deterred some laboratories from participating. The inherent limitations of surveys are apparent in the heterogeneity of the answers. Nevertheless, this report can serve as a baseline for more focused research on European laboratory capacity in the future.

Conclusion

The vision of an integrated Europe characterised by free movement of people, goods and services requires integrated systems to protect public health. The European Centre for Disease Prevention and Control is a key stakeholder in the development of integrated and harmonised systems. The European Surveillance System (TESSy) is the ECDC data collection system for EU surveillance data which collates data on the occurrence of diseases from public health agencies. A European network for surveillance of food- and waterborne diseases has been established by asking countries to nominate epidemiologists and laboratory experts for the six priority diseases in 2008. This European FWD Network forms the basis for core surveillance of food- and waterborne diseases at EU level. In addition to the case-based reporting from national institutes of public health, data from NRLs also form a central part of TESSy and contribute to the ECDC and Member States' capacity to detect and respond to European-wide threats to public health.

This survey of NRL capacity in the EU and EEA countries shows the diversity in the services available for some key food- and waterborne pathogens. Some countries have well developed comprehensive referral laboratories while others have little or none. While it is encouraging that many countries may have additional capacity with respect to one or all of these pathogens, the lack of minimum capacities in other Member States results in a non-standardised surveillance system which could hamper the early detection of and response to food-borne disease in a Europe-wide outbreak. There is a need for an accepted minimum level of referral laboratory capacity (in terms of scope and quality) for key pathogens throughout Europe to protect the health of all citizens. This is particularly important for FWD, given the continuing development of an effective single market in food products. This is also the reason why integration and collaboration of NRL services, firstly with national public health institutes and secondly with food safety authorities, are critical in recognising and managing infection related to contaminated food. Failure to address the lack of consistency in referral laboratory services (and more generally in surveillance systems) has the potential to create the mistaken impression that some countries with good surveillance systems have disproportionate problems with food- and waterborne disease when in fact they are simply detecting and reporting incidents that go unnoticed elsewhere.

In addition to addressing minimum requirements for referral laboratories in Member States it is appropriate to consider the value of developing European public health laboratory services, coordinated by ECDC, for those pathogens and diseases where this would be of added European value. Furthermore, there is room for strengthening ECDC cooperation with the WHO GFN and their Collaborating Centres where appropriate. The pace of change in laboratory methods and the convergence of characterisation methods on high-throughput molecular systems means that an overarching facility could provide an invaluable service for method development and harmonisation, to support quality improvement in national referral laboratories, provide technical support in outbreak situations and provide services related to infrequently isolated pathogens.

Bibliography

Buchholz U, Bernard H, Werber D, Böhmer M, Remschmidt C, Wilking D, et al. German outbreak of *Escherichia coli* O104:H4 Associated with sprouts." N Engl J Med 2011:365:1763-1770.

Centers for Disease Control and Prevention (CDC) Pulsenet 2012 Available at: http://www.cdc.gov/pulsenet/ [Accessed 25 June 2012]

Clinical and Laboratory Standards Institute (2009). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Eighth Edition. M07-A8. Jan 2009. Available at: http://www.clsi.org/source/orders/free/m07-a8.pdf [Accessed 25 June 2012]

European Centre for Disease Prevention and Control. Update of the position statement of the Commission and ECDC on human pathogen laboratories: A joint vision and strategy for the future. Stockholm, ECDC Management Board. Twenty-third Meeting. 9–10 November 2011.

European Centre for Disease Prevention and Control. Annual Epidemiological Report on Communicable Diseases in Europe 2010. ECDC. Stockholm 2010.

European Centre for Disease Prevention and Control. Core functions of microbiology reference laboratories for communicable diseases. ECDC. Stockholm June 2010.

European Centre for Disease Prevention and Control (2011). Risk assessment on the spread of carbapenemase-producing Enterobacteriaceae (CPE) through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. ECDC. Stockholm 2011.

European Centre for Disease Prevention and Control (2011). Updated risk assessment on the spread of New Delhi metallo-ß-lactamase (NDM) and its variants within Europe. ECDC. Stockholm 2011.

European Centre for Disease Prevention and Control. The European Surveillance System (TESSy). Available at: http://ecdc.europa.eu/en/activities/surveillance/tessy/pages/tessy.aspx [Accessed 25 June 2012]

European co-operation for Accreditation (EA). Available at: http://www.european-accreditation.org/content/home/home.htm [Accessed 25 June 2012]

European Commission Directorate-General for Health and Consumers. Official Controls - Reference laboratories - National Reference Laboratories (NRLs). Available at: http://ec.europa.eu/food/food/controls/reference_laboratories/national_reference_laboratories_en.htm [Accessed 25 June 2012]

European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antimicrobial susceptibility testing. Available at: http://www.eucast.org/antimicrobial_susceptibility_testing/ [Accessed 25 June 2012]

European Food Safety Authority. Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA Journal July 2008;732. [Last updated 4 June 2012]

European Food Safety Authority. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. EFSA Journal 2009: 223:221–215.

European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. EFSA Journal 2011;9(3):2090. Available at: www.efsa.europa.eu/efsajournal.

Frank C, Werber D, Cramer J, Askar M, Faber M, an der Heiden M et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. N Engl J Med 2011; 365(19):1771–1780.

O'Flanagan D, Cormican M, McKeown P, Nicolay N, Cowden J. Mason B et al. A multi-country outbreak of *Salmonella* Agona, February –August 2008. Euro Surveill 13(33).

Sheppard S, Dallas J, Strachan N, MacRae M, McCarthy N, Wilson D et al. *Campylobacter* genotyping to determine the source of human infection. Clin Infect Dis 2009;48(8):1072-1078.

Sprenger M. ECDC and the *Escherichia coli* outbreak in Germany. Lancet 2011; 377(9784):2180.

Tam C, Rodrigues L, Viviani L, Dodds J, Evans M, Hunter P et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 2011; 61(1):69–77.

Trachtman H. *Escherichia coli* O104:H4 outbreak in Germany. Author response. N Engl J Med 2012;366(8):766–767.

World Health Organization. International Health Regulations (2005). Available at http://www.who.int/ihr/en/ [Accessed 25 June 2012]

World Health Organization. Global Foodborne Infections Network (GFN). WHO 2012. Available at http://www.who.int/gfn/en/ [Accessed 25 June 2012]

Annexes: Sample survey forms

A1 Survey form for Campylobacter spp.

2. Contact information

21	Country name
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:
25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (public/private/other)
30	Contact person responsible for filling out the questionnaire
31	Position
32	Email
33	Phone

3. General information on the National Reference Laboratory performing *Campylobacter spp.* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *Campylobacter spp.* : *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of Campylobacter spp, then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by Non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	

	In which situation(s) do primary laboratories send isolates of *Campylobacter spp.* (or samples) to your laboratory ?	
317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	

	Does your laboratory also process *Campylobacter* isolates and/or samples from:	
323	Food	Y/N
324	Animals	Y/N
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable
	If Yes, please specify in which of the following areas:	
326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	Y/N/Not applicable
328	Typing methods	Y/N/Not applicable
329	Research	Y/N/Not applicable
C 330	Other (please specify in the space below):	

	Please, provide information on clinical sample volumes in 2008 by replying either to A or B question	
331A	Total number of human clinical samples cultured for *Campylobacter* in 2008?	
331B	**Estimated** total number of human clinical samples cultured for *Campylobacter* in 2008?	number
	Please, provide information on strain sample volumes in 2008 by replying either to A or B question	
332A	Total number of *Campylobacter* strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of *Campylobacter* strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

	4. Methods - *Campylobacter spp.*	
	Isolation/confirmation/identification	
	What methods does your laboratory use for identification and confirmation of *Campylobacter spp.*?	
41	Culture methods	Y/N
42	Non-culture-based methods	Yes (Enzyme Immuno Assay – EIA) Yes (PCR) Yes (other) No
C 43	**If you culture, please list below which media are routinely used to culture for *Campylobacter spp.*:**	
	Free text	
44	**Does your laboratory store the *Campylobacter spp.* strains (including the received strains) after examination ?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
45	Outbreak-related strains	Y/N/Not applicable
46	When specifically requested by the sending laboratory	Y
47	Randomly	N
C 48	Other (please specify in the space below):	
	Free text	
	Further characterisation/typing	
49	**Does your laboratory perform further characterisation/typing on *Campylobacter spp.* strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for *Campylobacter spp.* diagnostics?	
410	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
411	Species determination	
412	Serotyping	
413	Phage-typing	
414	**Molecular typing**	Yes (routinely) Yes (occasionally) No Not applicable
415	Flagellin gene restriction fragment length polymorphism (fla-PCR)	
416	Pulsed Field Gel Electrophoresis (PFGE) typing	
417	Ribotyping	
418	ERIC-RAPD	
419	Amplified Fragment Length Polymorphism (AFLP)	
420	Restriction Fragment Length Polymorphism (RFLP)	
421	Multilocus Sequence Typing (MLST)	
C 422	Other (please list below):	
	Free text	
423	**Does your laboratory plan to implement any (other) typing method for *Campylobacter spp.*?**	Y/N
C 424	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
	In which of the following case(s) is further characterisation/typing done?	
425	When specifically requested by the sending laboratory	Y/N/Not applicable
426	During outbreak investigations	
C 427	Other (please specify in the space below):	
	Free text	
428	**How many *Campylobacter spp.* strains from humans were examined in 2008?**	number
	How many of these strains were further examined for:	
429	Species determination	number
430	Additional molecular typing (RFLP, MLST, PFGE, etc…)	number
431	AST	number
	Antimicrobial Susceptibility Testing (AST)	
432	**Is there a national surveillance programme for *Campylobacter spp.* antimicrobial resistance in your country?**	Y/N
433	**Does your laboratory perform AST for *Campylobacter spp.*?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
434	During outbreak investigations	Y/N/Not applicable
435	When specifically requested by the sending laboratory	
C 436	Other (please specify in the space below):	
	Free text	
	When performing AST for *Campylobacter spp.* which method(s) do you use?	
437	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
438	Dilution (Minimum Inhibitory Concentration)	
439	E-Test method	
C 440	Other (please specify in the space below):	
	Free text	

When performing AST for *Campylobacter spp.* which antibiotics do you routinely test for?

441	Amoxicillin	Y/N/Not applicable
442	Amoxicillin – clavulanic acid	
443	Ampicillin	
444	Azithromycin	
445	Cefotaxime	
446	Chloramphenicol	
447	Ciprofloxacin	
448	Clindamycin	
449	Erythromycin	
450	Imipenem	
451	Gentamicin	
452	Nalidixic acid	
453	Tetracyclin	
C 454	Other (please list below):	
	Free text	

Which guidelines does your laboratory use to determine breakpoints/cut-off?

455	CLSI (formerly NCCLS)	Y/N/Not applicable
456	EUCAST	
C 457	Other (please specify full name in the space below):	
	Free text	
458	**Does your laboratory also identify antibiotic resistance genes in *Campylobacter spp.* by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) - *Campylobacter spp.*

		Yes/No
51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for *Campylobacter spp.*-related activities?**	Y/N
	Does your laboratory have an <u>internal</u> quality control programme which covers the following *Campylobacter spp.*-related activities?	
52	Isolation/identification/confirmation	Y/N
53	Species determination	
54	Serotyping	
55	Phage typing	
56	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any <u>external</u> quality control (EQA) scheme for the following *Campylobacter spp.*-related activities?

Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA Scheme Name	
57	Isolation/identification/confirmation	Y/N	Free text	C 58
59	Species determination			C 510
511	Serotyping			C 512
513	Phage typing			C 514
515	Antimicrobial Susceptibility Testing (AST)			C 516

Is your laboratory accredited for the following *Campylobacter spp.*-related activities?

517	Isolation/identification/confirmation	Y/N
518	Species determination	
519	Serotyping	
520	Phage typing	
521	Antimicrobial Susceptibility Testing (AST)	

522	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable
	If Yes, according to which standards is the laboratory accredited?	
524	ISO 17025	Y/N/Not applicable
525	ISO 15189	Y/N/Not applicable
C 526	Other (please specify in the space below):	

6. Training needs for *Campylobacter spp.*

		Yes/No
	Does the staff in your laboratory undergo regular training for the following *Campylobacter spp.*-related activities?	
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	

What are the training needs in your laboratory for the following areas? (only in relation to *Campylobacter spp.*)
Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality control/quality assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	
	Free text	

What kind of training format do you think would be most suitable to address your training needs? *Please rate from 1 (less suitable) to 5 (most suitable).*

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	
C 618	Other (please specify in the space below):	
	Free text	

7. Harmonisation needs for *Campylobacter spp.*

When processing *Campylobacter spp.* samples/strains, does your laboratory follow national or international recommendations for the following activities:
Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select 'N/A' (Not applicable) if there are no existing recommendations for the specific activity.

71	Isolation	Yes (International) Yes (National) No N/A
72	Confirmation	
73	Species determination	
74	Serotyping	
75	Phage typing	
76	Antimicrobial Susceptibility Testing (AST)	

77	**Does your laboratory provide guidelines and diagnostic procedures for *Campylobacter spp.* to primary laboratories in your country?**	Y/N

If Yes, please specify in which of the following areas:

720A	Identification/confirmation methods	Y/N/Not applicable
721A	Typing methods	
722A	AST methods	
723A	Bio-safety	
724A	Quality control/quality assurance	
725A	Accreditation	
C 726A	Other (please specify in the space below):	
	Free text	

C 78 **What kind of testing combination does your laboratory use for confirmation of *Campylobacter spp.*?**
Please describe in the space below
Free text

Does your laboratory provide any of the following reference services for *Campylobacter spp.* to other countries?

79	Isolation	Y/N
710	Confirmation	
711	Species determination	
712	Serotyping	
713	Phage typing	
714	Antimicrobial Susceptibility Testing (AST)	
C 715	Other *(please list below):*	
	Free text	

Does your laboratory use any of the following reference services for *Campylobacter spp.* provided by other countries?

716	Isolation	Y/N
717	Confirmation	
718	Species determination	
719	Serotyping	
720	Phage typing	
721	Antimicrobial Susceptibility Testing (AST)	
C 722	Other *(please list below):*	
	Free text	

Which molecular typing methods for *Campylobacter spp.* do you think would be most suitable for epidemiological surveillance purposes at the EU level?
Please rate from 1 (low priority) to 5 (high priority)

723	Flagellin gene restriction fragment length polymorphism (fla-PCR)	Number 1-5
724	Pulsed Field Gel Electrophoresis (PFGE) typing	
725	Ribotyping	
726	ERIC-RAPD	
727	Amplified Fragment Length Polymorphism (AFLP)	
728	Restriction Fragment Length Polymorphism (RFLP)	
729	Multilocus Sequence Typing (MLST)	
C 730	Other	

Please list full names below, including the rate value into brackets - e.g. method x (3).
Free text

Which of the following *Campylobacter spp.*-related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

731	Isolation	Number 1-5
732	Confirmation	
733	Species determination	
734	Serotyping	
735	Phage typing	
736	Antimicrobial Susceptibility Testing (AST)	
738	Virulence gene detection	
C 739	Other *(please list below):*	
	Free text	

A2 Survey form for Listeria monocytogenes

	2. Contact information
21	Country name
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:
25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (Public/Private/Other)
30	Contact person responsible for filling out the questionnaire
31	Position
32	Email
33	Phone

3. General information on the National Reference Laboratory performing *Listeria monocytogenes* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *L. monocytogenes*: *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of L. monocytogenes then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by Non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	
	In which situation(s) do primary laboratories send isolates of *L. monocytogenes* (or samples) to your laboratory?	
317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	
	Does your laboratory also process *L. monocytogenes* isolates and/or samples from:	
323	Food	Y/N
324	Animals	Y/N
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable
	If Yes, please specify in which of the following areas:	
326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	
328	Typing methods	
329	Research	
C 330	Other (please specify in the space below):	
	Free text	
	Please, provide information on clinical sample volumes in 2008 by replying either to A or B question	
331A	Total number of human clinical samples cultured for *L. monocytogenes* in 2008?	
331B	**Estimated** total number of human clinical samples cultured for *L. monocytogenes* in 2008?	number
	Please, provide information on strain sample volumes in 2008 by replying either to A or B question	
332A	Total number of *L. monocytogenes* strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of *L. monocytogenes* strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

4. Methods – *L. monocytogenes*

Isolation/confirmation/identification

	What methods does your laboratory use for identification and confirmation of *L. monocytogenes?*	
41	Culture methods	Y/N
42	Non-culture-based methods	Yes (Enzyme Immuno Assay – EIA) Yes (PCR) Yes (other) No
C 43	**If you culture, please list below which media are routinely used to culture for *L. monocytogenes***	
	Free text	
44	**Does your laboratory store the *L. monocytogenes* strains (including the received strains) after examination?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
45	Outbreak-related strains	Y/N/Not applicable
46	When specifically requested by the sending laboratory	
47	Randomly	
C 48	Other (please specify in the space below):	
	Free text	

Further characterisation/typing

49	**Does your laboratory perform further characterisation/typing on *L. monocytogenes* strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for *L. monocytogenes* diagnosis?	
411	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
412	Sorbitol fermentation	
413	Haemolysin production	
414	Serotyping by slide agglutination	
415	Serological identification (ELISA)	
416	Detection by nucleic acid-based procedures (e.g. PCR)	
417	Phage typing	
C 418	Other (please list below):	
	Free text	
419	**Molecular typing**	Yes (routinely) Yes (occasionally) No Not applicable
421	Pulsed Field Gel Electrophoresis (PFGE)	
422	Ribotyping	
423	Random Amplified Polymorphic DNA (RAPD)	
424	Multiple Loci VNTR Analysis (MLVA)	
C 428	Other (please list below):	
	Free text	
429	**Virulence gene(s) detection**	Yes (routinely) Yes (occasionally) No Not applicable
	In which of the following case(s) is further characterisation/typing done?	
430	When specifically requested by the sending laboratory	Y/N/Not applicable
431	During outbreak investigations	
C 432	Other (please specify in the space below):	
	Free text	
433	**Does your laboratory plan to implement any (other) typing method for *L. monocytogenes***	Y/N
C 434	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
	How many L. monocytogenes strains from humans were examined in 2008?	number
436	**How many of these strains were further characterised by molecular typing?**	number

Antimicrobial Susceptibility Testing (AST)

		Yes/No
437	**Is there a national surveillance programme for *L. monocytogenes* antimicrobial resistance in your country?**	Y/N
438	**Does your laboratory perform AST for *L. monocytogenes?***	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
439	During outbreak investigations	Y/N/Not applicable
440	When specifically requested by the sending laboratory	
C 441	Other (please specify in the space below):	
	Free text	
	When performing AST for *L. monocytogenes* which method(s) do you use?	
442	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
443	Dilution (Minimum Inhibitory Concentration)	
444	E-Test method	
C 445	Other (please specify in the space below):	
	Free text	

When performing AST for *L. monocytogenes* which antibiotics do you routinely test for?

446	Amoxicillin	Y/N/Not applicable
447	Amoxicillin – clavulanic acid	
448	Ampicillin	
449	Azithromycin	
450	Cefotaxime	
451	Chloramphenicol	
452	Ciprofloxacin	
453	Clindamycin	
454	Erythromycin	
455	Imipenem	
456	Gentamicin	
457	Nalidixic acid	
458	Tetracyclin	
C 459	Other (please list below):	
	Free text	

Which guidelines does your laboratory use to determine breakpoints/cut-off?

455	CLSI (formerly NCCLS)	Y/N/Not applicable
456	EUCAST	
C 457	Other (please specify full name in the space below):	

458	**Does your laboratory also identify antibiotic resistance genes in *L. monocytogenes* by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) – *L. monocytogenes*

51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for *L. monocytogenes* related activities?**	Y/N

Does your laboratory have an internal quality control programme which covers the following *L. monocytogenes* -related activities?

52	Identification/confirmation	Y/N
53	Serotyping	
54	Phage typing	
55	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any external quality control (EQA) scheme for the following *L. monocytogenes*-related activities?

Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA Scheme Name	
57	Identification/confirmation	Y/N	Free text	C 57
58	Serotyping			C 59
510	Phage typing			C 511
512	Antimicrobial Susceptibility Testing (AST)			C 513

Is your laboratory accredited for the following *L. monocytogenes*-related activities?

514	Identification/confirmation	Y/N
515	Typing	
516	Antimicrobial Susceptibility Testing (AST)	

517	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable

If Yes, according to which standards is the laboratory accredited?

519	ISO 17025	Y/N/Not applicable
520	ISO 15189	Y/N/Not applicable
C 521	Other (please specify in the space below):	

6. Training needs for *L. monocytogenes*

	Does the staff in your laboratory undergo regular training for the following *L. monocytogenes* -related activities?	**Yes/No**
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	

What are the training needs in your laboratory for the following areas? (only in relation to *L. monocytogenes)*

Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality control/quality assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	
	Free text	

What kind of training format do you think would be most suitable to address your training needs?

Please rate from 1 (less suitable) to 5 (most suitable).

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	
C 618	Other (please specify in the space below):	
	Free text	

7. Harmonisation needs for *L. monocytogenes*

When processing *L. monocytogenes* samples/strains, does your laboratory follow national or international recommendations for the following activities:

Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select N/A (Not applicable) if there are no existing recommendations for the specific activity

71	Isolation	Yes (International) Yes (National) No N/A
72	Confirmation	
73	Species determination	
74	Serotyping	
75	Phage typing	
76	Antimicrobial Susceptibility Testing (AST)	

77	**Does your laboratory provide guidelines and diagnostic procedures for *L. monocytogenes* to primary laboratories in your country?**	Y/N

If Yes, please specify in which of the following areas:

720A	Identification/confirmation methods	Y/N/Not applicable
721A	Typing methods	
722A	AST methods	
723A	Bio-safety	
724A	Quality control/quality assurance	
725A	Accreditation	
C 726A	Other (please specify in the space below):	
	Free text	

C 78 **What kind of testing combination does your laboratory use for confirmation of *L. monocytogenes?***

Please describe in the space below

Free text

Does your laboratory provide any of the following reference services for *L. monocytogenes* to other countries?

79	Isolation	Y/N
710	Confirmation	
711	Species determination	
712	Serotyping	
713	Phage typing	
714	Antimicrobial Susceptibility Testing (AST)	
C 715	Other *(please list below):*	
	Free text	

Does your laboratory use any of the following reference services for *L. monocytogenes* provided by other countries?

716	Isolation	Y/N
717	Confirmation	
718	Species determination	
719	Serotyping	
720	Phage typing	
721	Antimicrobial Susceptibility Testing (AST)	
C 722	Other *(please list below):*	
	Free text	

Which molecular typing methods for *L. monocytogenes* do you think would be most suitable for epidemiological surveillance purposes at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

724	Pulsed Field Gel Electrophoresis (PFGE) typing	
725	Ribotyping	
726	ERIC- Random Amplified Polymorphic DNA (RAPD)	
727	Multiple Loci VNTR Analysis (MLVA)	
C 731	Other *Please list full names below, including the rate value into brackets - e.g. method x (3).*	
	Free text	

Which of the following *L. monocytogenes* -related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

732	Isolation	Number 1-5
733	Confirmation	
734	Species determination	
735	Serotyping	
736	Phage typing	
737	Antimicrobial Susceptibility Testing (AST)	
738	Virulence gene detection	
C 739	Other *(please list below):*	
	Free text	

A3 Survey form for Salmonella spp.

	2. Contact information
21	Country name
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:
25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (public/private/other)
30	Contact person responsible for filling out the questionnaire
31	Position
32	Email
33	Phone

3. General information on the National Reference Laboratory performing *Salmonella spp.* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *Salmonella spp.*: *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of Salmonella spp. then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by Non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	
	In which situation(s) do primary laboratories send isolates of *Salmonella spp.* (or samples) to your laboratory?	
317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	
	Does your laboratory also process *Salmonella spp.* isolates and/or samples from:	
323	Food	Y/N
324	Animals	
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable
	If Yes, please specify in which of the following areas:	
326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	
328	Typing methods	
329	Research	
C 330	Other (please specify in the space below):	
	Please, provide information on clinical sample volumes in 2008 by replying either to A or B question	
331A	Total number of human clinical samples cultured for *Salmonella spp.* in 2008?	
331B	**Estimated** total number of human clinical samples cultured for *Salmonella spp.* in 2008?	number
	Please, provide information on strain sample volumes in 2008 by replying either to A or B question	
332A	Total number of *Salmonella spp.* strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of *Salmonella spp.* strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

4. Methods – *Salmonella spp.*

Isolation/confirmation/identification

	What methods does your laboratory use for identification and confirmation of *Salmonella spp.?*	
42	Culture methods	Y/N
43	Non-culture-based methods	
C 44	**If you culture, please list below which media are routinely used to culture for *Salmonella spp.?***	
	Free text	

45	**Does your laboratory store the *Salmonella spp.* strains (including the received strains) after examination?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
46	Outbreak-related strains	Y/N/Not applicable
47	When specifically requested by the sending laboratory	
48	Randomly	
C 49	Other (please specify in the space below):	
	Free text	

Further characterisation/typing

410	**Does your laboratory perform further characterisation/typing on *Salmonella spp.* strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for *Salmonella spp.* diagnosis?	
411	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
412	O antigens serotyping	
413	O and H antigens serotyping	
417	Phage typing	
C 418	Other (please list below):	
	Free text	
	Molecular typing	Yes (routinely) Yes (occasionally) No Not applicable
416	Pulsed Field Gel Electrophoresis (PFGE)	
417	Ribotyping	
418	Random Amplified Polymorphic DNA (RAPD)	
421	Amplified Fragment Length Polymorphism (AFLP)	
422	Multiple Loci VNTR Analysis (MLVA)	
423	Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)	
C 426	Other (please list below):	
	Free text	
427	**Virulence gene(s) detection**	Yes (routinely) Yes (occasionally) No Not applicable
	In which of the following case(s) is further characterisation/typing done?	
428	When specifically requested by the sending laboratory	Y/N/Not applicable
429	During outbreak investigations	
C 430	Other (please specify in the space below):	
	Free text	
431	**Does your laboratory plan to implement any (other) typing methods?**	Y/N
C 432	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
433	**How many *Salmonella spp.* strains from humans were examined in 2008?**	number
	How many of these strains were further examined for:	
434	Species determination	number
435	Additional molecular typing (RFLP, MLST, PFGE, etc…)	number
436	AST	number

Antimicrobial Susceptibility Testing (AST)

437	**Is there a national surveillance programme for *Salmonella spp.* antimicrobial resistance in your country?**	Y/N
438	**Does your laboratory perform AST for *Salmonella spp.?***	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
439	During outbreak investigations	Y/N/Not applicable
440	When specifically requested by the sending laboratory	
C 441	Other (please specify in the space below):	
	Free text	
	When performing AST for *Salmonella spp.* which method(s) do you use?	
442	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
443	Dilution (Minimum Inhibitory Concentration)	
444	E-Test method	
C 445	Other (please specify in the space below):	
	Free text	
	When performing AST for *Salmonella spp.* which antibiotics do you routinely test for?	
446	Amoxicillin	Y/N/Not applicable
447	Amoxicillin – clavulanic acid	
448	Ampicillin	
449	Azithromycin	
450	Cefotaxime	
451	Ceftazidime	
452	Cefuroxime	

453	Cephalothin	
454	Chloramphenicol	
455	Ciprofloxacin	
456	Clindamycin	
457	Erythromycin	
458	Imipenem	
459	Gentamicin	
460	Nalidixic acid	
461	Tetracyclin	
462	Trimethoprim-sulphamethoxazole	
C 463	Other (please list below):	
	Free text	

Which guidelines does your laboratory use to determine breakpoints/cut-off?

464	CLSI (formerly NCCLS)	Y/N/Not applicable
465	EUCAST	
C 466	Other (please specify full name in the space below):	
467	**Does your laboratory also identify antibiotic resistance genes in *Salmonella spp.* by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) – *Salmonella spp.*

51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for *Salmonella spp.* related activities?**	Y/N

Does your laboratory have an internal quality control programme which covers the following *Salmonella spp.*-related activities?

52	Isolation/identification/confirmation	Y/N
53	Species determination	
54	Serotyping	
55	Phage typing	
56	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any external quality control (EQA) scheme for the following *Salmonella spp.*-related activities?
Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA Scheme Name	
57	Isolation/identification/confirmation	Y/N	Free text	C 58
59	Species determination			C 510
511	Serotyping			C 512
513	Phage typing			C 514
515	Antimicrobial Susceptibility Testing (AST)			C 516

Is your laboratory accredited for the following *Salmonella spp.*-related activities?

517	Isolation/identification/confirmation	Y/N
518	Species determination	
519	Serotyping	
520	Phage typing	
521	Antimicrobial Susceptibility Testing (AST)	
522	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable

If Yes, according to which standards is the laboratory accredited?

524	ISO 17025	Y/N/Not applicable
525	ISO 15189	Y/N/Not applicable
C 526	Other (please specify in the space below):	

6. Training needs for *Salmonella spp.*

	Does the staff in your laboratory undergo regular training for the following *Salmonella spp.* -related activities?	**Yes/No**
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	

What are the training needs in your laboratory for the following areas? (only in relation to *Salmonella spp.)*
Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality Control/Quality Assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	

Free text

What kind of training format do you think would be most suitable to address your training needs?

Please rate from 1 (less suitable) to 5 (most suitable).

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	
C 618	Other (please specify in the space below):	

Free text

7. Harmonisation needs for *Salmonella spp.*

When processing *Salmonella spp.* samples/strains, does your laboratory follow national or international recommendations for the following activities:

Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select 'N/A'(Not applicable) if there are no existing recommendations for the specific activity

71	Isolation	Yes (international) Yes (national) No N/A
72	Confirmation	
73	Further characterisation/typing	
74	Antimicrobial Susceptibility Testing (AST)	
75	**Does your laboratory provide guidelines and diagnostic procedures for *Salmonella spp.* to primary laboratories in your country?**	Y/N

If Yes, please specify in which of the following areas:

720A	Identification/confirmation methods	Y/N/Not applicable
721A	Typing methods	
722A	AST methods	
723A	Bio-safety	
724A	Quality control/quality assurance	
725A	Accreditation	
C 726A	Other (please specify in the space below):	

Free text

C 76 **What kind of testing combination does your laboratory use for confirmation of *Salmonella spp.?***

Please describe in the space below

Free text

Does your laboratory provide any of the following reference services for *Salmonella spp.* to other countries?

77	Isolation	Y/N
78	Confirmation	
79	Further characterisation/typing	
710	Antimicrobial Susceptibility Testing (AST)	
C 711	Other *(please list below):*	

Free text

Does your laboratory use any of the following reference services for *Salmonella spp.* provided by other countries?

712	Isolation	Y/N
713	Confirmation	
714	Further characterisation/typing	
715	Antimicrobial Susceptibility Testing (AST)	
C 716	Other *(please list below):*	

Free text

Which molecular typing methods for *Salmonella spp.* do you think would be most suitable for epidemiological surveillance purposes at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

718	Pulsed Field Gel Electrophoresis (PFGE) typing	
719	Ribotyping	
720	Random Amplified Polymorphic DNA (RAPD)	
721	Amplified Fragment Length Polymorphism (AFLP)	
722	Multiple Loci VNTR Analysis (MLVA)	
723	Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)	
C 724	Other *Please list full names below, including the rate value into brackets - e.g. method x (3).*	

Free text

Which of the following *Salmonella spp.* -related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

725	Isolation	Number 1-5
726	Confirmation	
727	Phenotyping	
728	Serotyping	
729	Phage typing	
730	Virulence gene detection	
731	Antimicrobial Susceptibility Testing (AST)	
C 732	Other *(please list below):*	

Free text

A4 Survey form for Shigella spp.

	2. Contact information
21	Country name:
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:

Please provide the following details:

25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (public/private/other)
210	Contact person responsible for filling out the questionnaire
211	Position
212	Email
213	Phone

3. General information on the National Reference Laboratory performing *Shigella spp.* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *Salmonella spp.*: *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of Salmonella spp. then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by Non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	

In which situation(s) do primary laboratories send isolates of *Shigella spp.* (or samples) to your laboratory?

317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	

Does your laboratory also process *Shigella spp.* isolates and/or samples from:

323	Food	Y/N
324	Animals	Y/N
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable

If Yes, please specify in which of the following areas:

326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	
328	Typing methods	
329	Research	
C 330	Other (please specify in the space below):	

Please, provide information on clinical sample volumes in 2008 by replying either to A or B question

331A	Total number of human clinical samples cultured for *Shigella spp.* in 2008?	
331B	**Estimated** total number of human clinical samples cultured for *Shigella spp.* in 2008?	number

Please, provide information on strain sample volumes in 2008 by replying either to A or B question

332A	Total number of *Shigella spp.* strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of *Shigella spp.* strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

4. Methods – *Shigella spp.*

Isolation/confirmation/identification

What methods does your laboratory use for identification and confirmation of *Salmonella spp.?*

42	Culture methods	Y/N
43	Non-culture-based methods	
C 44	**If you culture, please list below which media are routinely used to culture for *Shigella spp.?***	
	Free text	
45	**Does your laboratory store the *Shigella spp.* strains (including the received strains) after examination?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
46	Outbreak-related strains	Y/N/Not applicable
47	When specifically requested by the sending laboratory	
48	Randomly	
C 49	Other (please specify in the space below):	
	Free text	

Further characterisation/typing

410	**Does your laboratory perform further characterisation/typing on *Shigella spp.* strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for *Shigella spp.* diagnosis?	
411	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
412	Species determination using biochemical tests	
413	Species determination by slide agglutination with polyvalent antisera (A, B,C,D)	
414	Serotyping by slide agglutination with monovalent O-specific antisera	
415	Phage typing	
416	**Molecular typing**	Yes (routinely) Yes (occasionally) No Not applicable
418	Pulsed Field Gel Electrophoresis (PFGE)	
419	Ribotyping	
420	Plasmid profile analysis	
423	Multilocus Sequence Typing (MLST)	
424	Enterobacterial Repetitive Intergenic Consensus (ERIC)	
C 425	Other (please list below):	
	Free text	
426	**Virulence gene(s) detection**	Yes (routinely) Yes (occasionally) No Not applicable
	In which of the following case(s) is further characterisation/typing done?	
427	When specifically requested by the sending laboratory	Y/N/Not applicable
428	During outbreak investigations	
C 429	Other (please specify in the space below):	
	Free text	
430	**Does your laboratory plan to implement any (other) typing methods?**	Y/N
C 431	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
432	**How many *Shigella spp.* strains from humans were examined in 2008?**	number
	How many of these strains were further examined for:	
433	Species determination	number
434	Additional molecular typing (RFLP, MLST, PFGE, etc…)	number
435	Antimicrobial susceptibility testing (AST)	number

Antimicrobial Susceptibility Testing (AST)

436	**Is there a national surveillance programme for *Shigella spp.* antimicrobial resistance in your country?**	Y/N
437	**Does your laboratory perform AST for *Shigella spp.?***	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
438	During outbreak investigations	Y/N/Not applicable
439	When specifically requested by the sending laboratory	
C 440	Other (please specify in the space below):	
	Free text	
	When performing AST for *Shigella spp.* which method(s) do you use?	
441	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
442	Dilution (Minimum Inhibitory Concentration)	
443	E-Test method	
C 444	Other (please specify in the space below):	
	Free text	
	When performing AST for *Shigella spp.* which antibiotics do you routinely test for?	
445	Amoxicillin	Y/N/Not applicable
446	Amoxicillin – clavulanic acid	
447	Ampicillin	

448	Azithromycin	
449	Cefotaxime	
450	Chloramphenicol	
451	Ciprofloxacin	
452	Clindamycin	
453	Erythromycin	
454	Imipenem	
455	Gentamicin	
456	Nalidixic acid	
457	Tetracyclin	
C 458	Other (please list below):	
	Free text	

Which guidelines does your laboratory use to determine breakpoints/cut-off?

459	CLSI (formerly NCCLS)	
460	EUCAST	
C 461	Other (please specify full name in the space below):	
462	**Does your laboratory also identify antibiotic resistance genes in *Shigella spp.* by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) – *Shigella spp.*

51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for *Shigella spp.* related activities?**	Y/N

Does your laboratory have an internal quality control programme which covers the following *Shigella spp.*-related activities?

52	Identification/confirmation	Y/N
53	Species determination	
54	Serotyping	
55	Phage typing	
56	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any external quality control (EQA) scheme for the following *Shigella spp.*-related activities?

Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA Scheme Name	
57	Identification/confirmation	Y/N	Free text	C 58
59	Species determination			C 510
511	Serotyping			C 512
513	Phage typing			C 514
515	Antimicrobial Susceptibility Testing (AST)			C 516

Is your laboratory accredited for the following *Shigella spp.*-related activities?

517	Identification/confirmation	Y/N
518	Species determination	
519	Serotyping	
520	Phage typing	
521	Antimicrobial Susceptibility Testing (AST)	
522	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable

If Yes, according to which standards is the laboratory accredited?

524	ISO 17025	Y/N/Not applicable
525	ISO 15189	
C 526	Other (please specify in the space below):	

6. Training needs for *Shigella spp.*

	Does the staff in your laboratory undergo regular training for the following *Shigella spp.* -related activities?	**Yes/No**
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	
	Free text	

What are the training needs in your laboratory for the following areas? (only in relation to *Shigella spp.)*

Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality control/quality assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	

What kind of training format do you think would be most suitable to address your training needs? *Please rate from 1 (less suitable) to 5 (most suitable).*

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	
C 618	Other (please specify in the space below):	
	Free text	

7. Harmonisation needs for *Shigella spp.*

When processing *Shigella spp.* samples/strains, does your laboratory follow national or international recommendations for the following activities:

Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select 'N/A' (Not applicable) if there are no existing recommendations for the specific activity

71	Isolation	Yes (International) Yes (National) No N/A
72	Confirmation	
73	Further characterisation/typing	
74	Antimicrobial Susceptibility Testing (AST)	
75	**Does your laboratory provide guidelines and diagnostic procedures for *Shigella spp.* to primary laboratories in your country?**	Y/N

If Yes, please specify in which of the following areas:

720A	Identification/confirmation methods	Y/N/Not applicable
721A	Typing methods	
722A	AST methods	
723A	Bio-safety	
724A	Quality control/quality assurance	
725A	Accreditation	
C 726A	Other (please specify in the space below):	
	Free text	
C 76	**What kind of testing combination does your laboratory use for confirmation of *Shigella spp.?*** *Please describe in the space below*	
	Free text	

Does your laboratory provide any of the following reference services for *Shigella spp.* to other countries?

77	Isolation	Y/N
78	Confirmation	
79	Further characterisation/typing	
710	Antimicrobial Susceptibility Testing (AST)	
C 711	Other *(please list below):*	
	Free text	

Does your laboratory use any of the following reference services for *Shigella spp.* provided by other countries?

712	Isolation	Y/N
713	Confirmation	
714	Further characterisation/typing	
715	Antimicrobial Susceptibility Testing (AST)	
C 716	Other *(please list below):*	
	Free text	

Which molecular typing methods for *Shigella spp.* do you think would be most suitable for epidemiological surveillance purposes at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

718	Pulsed Field Gel Electrophoresis (PFGE) typing	
719	Ribotyping	
720	Plasmid profile analysis	
721	Multilocus Sequence Typing (MLST)	
722	Enterobacterial Repetitive Intergenic Consensus (ERIC)	
C 724	Other: *Please list full names below, including the rate value into brackets - e.g. method x (3).*	
	Free text	

Which of the following *Shigella spp.* -related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

725	Isolation	Number 1-5
726	Confirmation	
727	Phenotyping	
728	Serotyping	
729	Phage typing	
730	Virulence gene detection	
731	Antimicrobial Susceptibility Testing (AST)	
C 732	Other *(please list below):*	
	Free text	

A5 Survey form for STEC/VTEC

	2. Contact information
21	Country name:
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:

Please provide the following details:

25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (public/private/other)
210	Contact person responsible for filling out the questionnaire
211	Position
212	Email
213	Phone

3. General information on the National Reference Laboratory performing *Verocytotoxin-producing Escherichia coli (STEC/VTEC)* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *STEC/VTEC:* *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of STEC/VTEC. then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	
	In which situation(s) do primary laboratories send isolates of STEC/VTEC (or samples) to your laboratory?	
317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	
	Does your laboratory also process STEC/VTEC isolates and/or samples from:	
323	Food	Y/N
324	Animals	
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable
	If Yes, please specify in which of the following areas:	
326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	
328	Typing methods	
329	Research	
C 330	Other (please specify in the space below):	
	Please, provide information on clinical sample volumes in 2008 by replying either to A or B question	
331A	Total number of human clinical samples cultured for STEC/VTEC in 2008?	
331B	**Estimated** total number of human clinical samples cultured for STEC/VTEC in 2008?	number
	Please, provide information on strain sample volumes in 2008 by replying either to A or B question	
332A	Total number of STEC/VTEC strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of STEC/VTEC strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

4. Methods – STEC/VTEC

Isolation/confirmation/identification

	What methods does your laboratory use for identification and confirmation of STEC/VTEC?	
41	Culture methods	Yes (routinely) Yes (occasionally) No
42	Non-culture-based methods	
43	Serology	
44	PCR	
C 45	**If you culture, please list below which media are routinely used to culture for STEC/VTEC?**	
	Free text	

46	**Does your laboratory store the STEC/VTEC strains (including the received strains) after examination?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
47	Outbreak-related strains	Y/N/Not applicable
48	When specifically requested by the sending laboratory	
49	Randomly	
C 50	Other (please specify in the space below):	
	Free text	
	Further characterisation/typing	
411	**Does your laboratory perform further characterisation/typing on STEC/VTEC strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for STEC/VTEC diagnosis?	
412	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
413	Sorbitol fermentation	
414	Beta Glucorinidase production	
415	Haemolysin production	
416	Phage typing	
C 417	Other (please list below):	
	Free text	
418	**Verocytotoxin testing**	Yes (routinely) Yes (occasionally) No Not applicable
419	Vero Cell Assay	
420	EIA	
421	Toxin gene detection	
422	**Molecular typing**	Yes (routinely) Yes (occasionally) No Not applicable
424	Pulsed Field Gel Electrophoresis (PFGE)	
426	Random Amplified Polymorphic DNA (RAPD)	
427	Multiple Loci VNTR Analysis (MLVA)	
428	Restriction Fragment Length Polymorphism (RFLP)	
428-1	Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)	
429	Multi Locus Sequence Typing (MLST)	
C 430	Other (please list below):	
	Free text	
431	**O Grouping**	Yes (routinely) Yes (occasionally) No Not applicable
432	Full range (01 through O181)	
C 433	Selected O groups only (please list below)	
	Free text	
434	**Phenotypic O grouping**	Yes (routinely) Yes (occasionally) No Not applicable
435	Slide agglutination	
436	Tube agglutination	
	Free text	
437	**Genotypic O grouping**	Yes (routinely) Yes (occasionally) No Not applicable
438	PCR	
439	Sequencing	
440	RFLP	
C 441	Other (please list below):	
	Free text	
442	**H Typing**	Yes (routinely) Yes (occasionally) No Not applicable
443	Full range (H1 through H53)	
C 444	Selected H antigens only (please list below):	
	Free text	
445	**Phenotypic H Typing**	Yes (routinely) Yes (occasionally) No Not applicable
446	**Genotypic H Typing**	Yes (routinely) Yes (occasionally) No Not applicable
447	PCR	
448	Sequencing	
449	RFLP	
450	fliC RFLP	
C 451	Other (please list below):	
	Free text	
452	**Virulence gene(s) detection**	Yes (routinely) Yes (occasionally) No Not applicable
	If Yes, which of the following virulence genes do you test for?	
453	Verocytotoxin 1 gene (vtx1)	Y/N
454	Verocytotoxin 2 gene (vtx2)	

455	Intimin gene (eae)	
456	Haemolysin A gene (ehxA)	
	If Yes, do you perform subtyping of the following virulence genes?	
457	Verocytotoxin 1 gene (vtx1)	Y/N
458	Verocytotoxin 2 gene (vtx2)	
459	Intimin gene (eae)	
460	Haemolysin A gene (ehxA)	
	In which of the following case(s) is further characterisation/typing done?	
461	When specifically requested by the sending laboratory	Y/N/Not applicable
462	During outbreak investigations	
C 463	Other (please specify in the space below):	
	Free text	
464	**Does your laboratory plan to implement any (other) typing methods?**	Y/N
C 465	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
466	**How many STEC/VTEC strains from humans were examined in 2008?**	number
467	**How many of these strains were further characterised by molecular typing?**	number

Antimicrobial Susceptibility Testing (AST)

468	**Is there a national surveillance programme for STEC/VTEC antimicrobial resistance in your country?**	Y/N
469	**Does your laboratory perform AST for STEC/VTEC?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
470	Routinely for all received and/or isolated strains	Y/N/Not applicable
471	During outbreak investigations	
472	When specifically requested by the sending laboratory	
C 473	Other (please specify in the space below):	
	Free text	
	When performing AST for STEC/VTEC which method(s) do you use?	
474	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
475	Dilution (Minimum Inhibitory Concentration)	
476	E-Test method	
C 477	Other (please specify in the space below):	
	Free text	
	When performing AST for STEC/VTEC which antibiotics do you routinely test for?	
478	Amoxicillin	Y/N/Not applicable
479	Amoxicillin – clavulanic acid	
480	Ampicillin	
481	Azithromycin	
482	Cefotaxime	
483	Chloramphenicol	
484	Ciprofloxacin	
485	Clindamycin	
486	Erythromycin	
487	Imipenem	
488	Gentamicin	
489	Nalidixic acid	
490	Tetracyclin	
C 491	Other (please list below):	
	Free text	
	Which guidelines does your laboratory use to determine breakpoints/cut-off?	
492	CLSI (formerly NCCLS)	Y/N/Not applicable
493	EUCAST	
C 494	Other (please specify full name in the space below):	
495	**Does your laboratory also identify antibiotic resistance genes in STEC/VTEC by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) – STEC/VTEC

51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for STEC/VTEC related activities?**	Y/N
	Does your laboratory have an <u>internal</u> quality control programme which covers the following STEC/VTEC related activities?	
52	Identification/confirmation	Y/N
53	Typing	
54	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any <u>external</u> quality control (EQA) scheme for the following STEC/VTEC-related activities?

Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA Scheme Name	
55	Identification/confirmation	Y/N	Free text	C 56
57	Typing			C 58
59	Antimicrobial Susceptibility Testing (AST)			C 510

Is your laboratory accredited for the following STEC/VTEC-related activities?

517	Identification/confirmation	Y/N
518	Typing	
521	Antimicrobial Susceptibility Testing (AST)	
522	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable
	If Yes, according to which standards is the laboratory accredited?	
516	ISO 17025	Y/N/Not applicable
517	ISO 15189	
C 518	Other (please specify in the space below):	

6. Training Needs for STEC/VTEC

	Does the staff in your laboratory undergo regular training for the following STEC/VTEC -related activities?	**Yes/No**
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	
	Free text	

What are the training needs in your laboratory for the following areas? (only in relation to STEC/VTEC)

Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality control/quality assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	
	Free text	

What kind of training format do you think would be most suitable to address your training needs? *Please rate from 1 (less suitable) to 5 (most suitable).*

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	
C 618	Other (please specify in the space below):	
	Free text	

7. Harmonisation needs for STEC/VTEC

When processing STEC/VTEC samples/strains, does your laboratory follow national or international recommendations for the following activities:

Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select 'N/A'(Not applicable) if there are <u>no existing recommendations</u> for the specific activity

71	Isolation	Yes (International) Yes (National) No N/A
72	Confirmation	
73	Further characterisation/typing	
74	Antimicrobial Susceptibility Testing (AST)	
75	**Does your laboratory provide guidelines and diagnostic procedures for STEC/VTEC to primary laboratories in your country?**	Y/N
	If Yes, please specify in which of the following areas:	
720A	Identification/confirmation methods	Y/N/Not applicable
721A	Typing methods	
722A	AST methods	
723A	Bio-safety	
724A	Quality Control/Quality Assurance	
725A	Accreditation	
C 726A	Other (please specify in the space below):	
	Free text	
C 76	**What kind of testing combination does your laboratory use for confirmation of STEC/VTEC?** *Please describe in the space below*	
	Free text	
	Does your laboratory provide any of the following reference services for STEC/VTEC to other countries?	
77	Isolation	Y/N
78	Confirmation	
79	Further characterisation/Typing	
710	Antimicrobial Susceptibility Testing (AST)	
C 711	Other *(please list below):*	
	Free text	
	Does your laboratory use any of the following reference services for STEC/VTEC provided by other countries?	
712	Isolation	Y/N
713	Confirmation	
714	Further characterisation/Typing	
715	Antimicrobial Susceptibility Testing (AST)	
C 716	Other *(please list below):*	

Which molecular typing methods for STEC/VTEC do you think would be most suitable for epidemiological surveillance purposes at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

717	Pulsed Field Gel Electrophoresis (PFGE) typing	Number 1-5
718	Random Amplified Polymorphic DNA (RAPD)	
729	Multiple Loci VNTR Analysis (MLVA)	
720	Restriction Fragment Length Polymorphism (RFLP)	
721	Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)	
722	Multi Locus Sequence Typing (MLST)	
C 724	Other: *Please list full names below, including the rate value into brackets - e.g. method x (3).*	
	Free text	

Which of the following STEC/VTEC -related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

725	Isolation	Number 1-5
726	Confirmation	
727	Phenotyping	
728	Serotyping	
729	Phage typing	
730	Virulence gene detection	
731	Verocytotoxin detection	
732	Antimicrobial Susceptibility Testing (AST)	
C 733	Other *(please list below):*	
	Free text	

A6 Survey form for Yersinia spp.

	2. Contact information
21	Country name:
22	Contact Point name:
23	Alternate Contact Point (if any) name:
24	Date form completed:

Please provide the following details:

25	Institution
26	Laboratory name
27	Name in local language
28	Address
29	Status (public/private/other)
210	Contact person responsible for filling out the questionnaire
211	Position
212	Email
213	Phone

3. General information on the National Reference Laboratory performing *Yersinia spp.* microbiology

	Please indicate which of the following activities do apply to your laboratory in relation to *Yersinia spp.*: *(e.g. if your laboratory performs 'isolation and confirmation' of other pathogens but not of Salmonella spp. then answer 'No' here)*	**Yes/No**
31	Isolation	Y/N
32	Identification/confirmation by Non-culture-based methods	
33	Further typing	
34	Antimicrobial Susceptibility Testing (AST)	
35	Training	
36	Microbiological advice (e.g. by emails/phone)	
37	Method development	
38	Organising ring trials	
39	Providing material (e.g. strains, samples) for proficiency testing organisers	
310	Research & scientific publications	
311	Support in outbreak investigations	
312	Support in quality assurance for primary and regional (local) laboratories	
313	Provision of reference material (e.g. reference strains) to the laboratories	
314	Maintaining a strain collection	
315	Providing guidelines and diagnostic procedures for primary laboratories in your country	
316	Developing proposals for the standardisation of methods	

	In which situation(s) do primary laboratories send isolates of *Yersinia spp.* (or samples) to your laboratory?	
317	All isolates are sent to your laboratory	Y/N
318	Diagnostic reasons *(for example, to confirm the isolation)*	
319	Outbreak investigation	
320	Defined proportion of isolates at regular time intervals	
321	Randomly - no regular intervals, no specific reasons	
C 322	Other (please specify in the space below):	
	Free text	

	Does your laboratory also process *Yersinia spp.* isolates and/or samples from:	
323	Food	Y/N
324	Animals	
325	**Does your laboratory have a working collaboration with the national institute of public health in your country?**	Y/N/Not applicable
	If Yes, please specify in which of the following areas:	
326	Surveillance	Y/N/Not applicable
327	Outbreak investigation	
328	Typing methods	
329	Research	
C 330	Other (please specify in the space below):	

	Please, provide information on clinical sample volumes in 2008 by replying either to A or B question	
331A	Total number of human clinical samples cultured for *Yersinia spp.* in 2008?	
331B	**Estimated** total number of human clinical samples cultured for *Yersinia spp.* in 2008?	number
	Please, provide information on strain sample volumes in 2008 by replying either to A or B question	
332A	Total number of *Yersinia spp.* strains confirmed or further characterised in 2008?	
332B	**Estimated** total number of *Yersinia spp.* strains confirmed or further characterised in 2008?	number
C 333	Please add comments on confirmation or further characterisation if you think necessary:	
	Free text	

4. Methods – *Yersinia spp.*

Isolation/confirmation/identification

	What methods does your laboratory use for identification and confirmation of *Yersinia spp.?*	
41	Culture methods	Y/N
42	Non-culture-based methods	
C 43	**If you culture, please list below which media are routinely used to culture for *Yersinia spp.?***	
	Free text	

44	**Does your laboratory store the *Yersinia spp.* strains (including the received strains) after examination?**	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) are strains stored?	
45	Outbreak-related strains	Y/N/Not applicable
46	When specifically requested by the sending laboratory	
47	Randomly	
C 48	Other (please specify in the space below):	
	Free text	

Further characterisation/typing

411	**Does your laboratory perform further characterisation/typing on *Yersinia spp.* strains?**	Yes (routinely) Yes (occasionally) No
	If Yes, which of the following methods does your laboratory use for *Yersinia spp.* diagnosis?	
412	**Phenotyping**	Yes (routinely) Yes (occasionally) No Not applicable
413	Biotyping	
414	Species determination	
415	Serotyping	
416	Phage typing	
C 417	Other (please list below):	
	Free text	
418	**Molecular typing**	Yes (routinely) Yes (occasionally) No Not applicable
420	Pulsed Field Gel Electrophoresis (PFGE)	
421	Ribotyping	
422	Random Amplified Polymorphic DNA (RAPD)	
425	Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)	
426	Multiple Loci VNTR Analysis (MLVA)	
427	Multilocus Sequence Typing (MLST)	
C 427-1	Other (please list below):	
	Free text	
428	**Virulence gene(s) detection**	Yes (routinely) Yes (occasionally) No Not applicable
432	**Does your laboratory plan to implement any (other) typing methods?**	Y/N
C 433	**If yes, please specify what typing method(s) is planned to be implemented?**	
	Free text	
	How many *Yersinia spp.* strains from humans were examined in 2008?	
434	*Yersinia* enterocolitica	number
435	*Yersinia* pseudotuberculosis	number
	How many *Y. enterocolitica* strains were further examined for:	
436	Additional molecular typing (RFLP, MLST, PFGE, etc…)	number
437	Antimicrobial susceptibility testing (AST)	number
	How many *Y. pseudotuberculosis* strains were further examined for:	
439	Additional molecular typing (RFLP, MLST, PFGE, etc…)	number
440	Antimicrobial susceptibility testing (AST)	number

Antimicrobial Susceptibility Testing (AST)

		Yes/No
441	**Is there a national surveillance programme for *Yersinia spp.* antimicrobial resistance in your country?**	Y/N
442	**Does your laboratory perform AST for *Yersinia spp.?***	Yes (routinely) Yes (occasionally) No
	If Yes, in which of the following case(s) is AST performed?	
443	During outbreak investigations	Y/N/Not applicable
444	When specifically requested by the sending laboratory	
C 445	Other (please specify in the space below):	
	Free text	
	When performing AST for *Yersinia spp.* which method(s) do you use?	
446	Disc diffusion	Yes (routinely) Yes (occasionally) No Not applicable
447	Dilution (Minimum Inhibitory Concentration)	
448	E-Test method	
C 449	Other (please specify in the space below):	
	Free text	
	When performing AST for *Yersinia spp.* which antibiotics do you routinely test for?	
450	Amoxicillin	Y/N/Not applicable
451	Amoxicillin – clavulanic acid	
452	Ampicillin	
453	Azithromycin	
454	Cefotaxime	
455	Chloramphenicol	
456	Ciprofloxacin	
457	Clindamycin	

458	Erythromycin	
459	Imipenem	
460	Gentamicin	
461	Nalidixic acid	
462	Tetracyclin	
C 463	Other (please list below):	
	Free text	

Which guidelines does your laboratory use to determine breakpoints/cut-off?

464	CLSI (formerly NCCLS)	Y/N/Not applicable
465	EUCAST	
C 466	Other (please specify full name in the space below):	

467	**Does your laboratory also identify antibiotic resistance genes in *Yersinia spp.* by molecular techniques?**	Yes (routinely) Yes (occasionally) No Not applicable

5. Quality control and external quality assurance (EQA) – *Yersinia spp.*

51	**Does your laboratory participate in regular proficiency testing programmes and/or inter-laboratory comparisons for *Yersinia spp.* related activities?**	Y/N

Does your laboratory have an internal quality control programme which covers the following *Yersinia spp.*-related activities?

52	Isolation/identification/confirmation	Y/N
53	Species determination	
54	Serotyping	
55	Phage typing	
56	Antimicrobial Susceptibility Testing (AST)	

Does your laboratory participate in any external quality control (EQA) scheme for the following *Yersinia spp.*-related activities?

Select Y/N and, if Yes, please specify the name of the scheme in the right column.

			EQA scheme name	
57	Isolation/identification/confirmation	Y/N	Free text	C 58
59	Species determination			C 510
511	Serotyping			C 512
513	Phage typing			C 514
515	Antimicrobial Susceptibility Testing (AST)			C 516

Is your laboratory accredited for the following *Yersinia spp.*-related activities?

517	Isolation/identification/confirmation	Y/N
518	Species determination	
519	Serotyping	
520	Phage typing	
521	Antimicrobial Susceptibility Testing (AST)	

522	**If Yes, which type of accreditation body does provide the accreditation (national/international)?**	Y/N/Not applicable

If Yes, according to which standards is the laboratory accredited?

524	ISO 17025	Y/N/Not applicable
525	ISO 15189	Y/N/Not applicable
C 526	Other (please specify in the space below):	
	Free text	

6. Training needs for *Yersinia spp.*

	Does the staff in your laboratory undergo regular training for the following *Yersinia spp.* -related activities?	**Yes/No**
61	Identification/confirmation methods	Y/N
62	Typing methods	
63	AST methods	
64	Bio-safety	
65	Quality control/quality assurance	
66	Accreditation	
C 67	Other (please specify in the space below):	
	Free text	

What are the training needs in your laboratory for the following areas? (only in relation to *Yersinia spp.)*

Please rate from 1 (low priority) to 5 (high priority) the following listed areas

68	Identification/confirmation methods	Number 1-5
69	Typing methods	
610	AST methods	
611	Bio-safety	
612	Quality control/quality assurance	
613	Accreditation	
C 614	Other (please specify in the space below):	
	Free text	

What kind of training format do you think would be most suitable to address your training needs? *Please rate from 1 (less suitable) to 5 (most suitable).*

615	Short courses	Number 1-5
616	Online training	
617	Hands-on training	

C 618 Other (please specify in the space below):

7. Harmonisation needs for *Yersinia spp.* (excluding Y. pestis) activities

When processing *Yersinia spp.* samples/strains, does your laboratory follow national or international recommendations for the following activities:

Please select 'No' if your laboratory does not use any of the national/international recommendations available, and select 'N/A' (Not applicable) if there are no existing recommendations for the specific activity

71	Isolation	Yes (International) Yes (National) No N/A
72	Confirmation	
73	Further characterisation/typing	
74	Antimicrobial Susceptibility Testing (AST)	
75	**Does your laboratory provide guidelines and diagnostic procedures for *Yersinia spp.* to primary laboratories in your country?**	Y/N
	If Yes, please specify in which of the following areas:	
76	Identification/confirmation methods	Y/N/Not applicable
77	Typing methods	
78	AST methods	
79	Bio-safety	
710	Quality control/quality assurance	
711	Accreditation	
C 712	Other (please specify in the space below):	

Free text

C 713 **What kind of testing combination does your laboratory use for confirmation of *Yersinia spp.?*** *Please describe in the space below*

Free text

Does your laboratory provide any of the following reference services for *Yersinia spp.* to other countries?

714	Isolation	Y/N
715	Confirmation	
716	Further characterisation/typing	
717	Antimicrobial Susceptibility Testing (AST)	
C 718	Other *(please list below):*	

Free text

Does your laboratory use any of the following reference services for *Yersinia spp.* provided by other countries?

719	Isolation	Y/N
720	Confirmation	
721	Further characterisation/typing	
722	Antimicrobial Susceptibility Testing (AST)	
C 723	Other *(please list below):*	

Free text

Which molecular typing methods for *Yersinia spp.* do you think would be most suitable for epidemiological surveillance purposes at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

724	Pulsed Field Gel Electrophoresis (PFGE) typing	Y/N
725	Ribotyping	
726	Random Amplified Polymorphic DNA (RAPD)	
727	Enterobacterial Repetitive Intergenic Consensus (ERIC)	
728	Multiple Loci VNTR Analysis (MLVA)	
729	Multilocus Sequence Typing (MLST)	
C 732	Other	

Please list full names below, including the rate value into brackets - e.g. method x (3).

Free text

Which of the following *Yersinia spp.* -related activities do you think would require method harmonisation at the EU level?

Please rate from 1 (low priority) to 5 (high priority)

733	Isolation	Number 1-5
734	Confirmation	
735	Species determination	
736	Serotyping	
737	Phage typing	
738	Antimicrobial Susceptibility Testing (SAT)	
739	Virulence gene detection	
C 740	Other *(please list below):*	

Free text