



Public Health
England

Protecting and improving the nation's health

Report of UK National Reference Laboratory for Food Microbiology

Activities for *Listeria monocytogenes*,
coagulase positive staphylococci,
Escherichia coli (including STEC),
Campylobacter, *Salmonella* and
antimicrobial resistance

April 2018 to March 2019

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Executive summary

Public Health England provides the UK's National Reference Laboratory (NRL) for food microbiology for the Food Standards Agency (FSA), as part of the UK's compliance to the Regulations (EU) 882/2004 and 2017/625 for official controls for food safety. This is the annual report of the NRL's activities between April 2018 and March 2019 and relates to activities for *Listeria monocytogenes*, coagulase positive staphylococci, *Escherichia coli* (incl. STEC), *Campylobacter*, *Salmonella* and antimicrobial resistance (AR).

European Reference Laboratory (EURL) information and NRL quarterly newsletters were disseminated to the FSA, the Official Control Laboratories (OCLs) and other stakeholders. To inform OCLs of any developments from the EURLs, legislation changes and methodology updates, an annual OCL user day was held. The UK Food Examiner register was also reviewed to maintain current records. The NRL also advised and contributed of their position regarding EU Exit to other departments in PHE, Defra and FSA.

All 6 EURL meetings were attended by the UK NRL, and impartial advice was provided to FSA, OCLs and other laboratories throughout the year. The NRL also attended EURL training for detection and characterisation of STEC from food by ISO/TS 13136. The FSA is undergoing an Official Laboratories review, of which the NRL has given information for both parts of this process. The NRL is active in the BSI AW9 microbiology committee and is a member of the CEN TAG18 expert working group for the revision of the ISO TS 13136 (PCR detection of shiga toxin-producing *Escherichia coli*).

Food methods are provided by the NRL to OCLs via the gov.uk website. Most have had impact assessments performed after EU Mandate 381 required all ISO Standards that are referenced in the Microbiological Criteria EU 2073/2015 were revised.

Thirteen OCLs participated in the European Food Microbiology Legislation (EFL) External Quality Assessment Scheme, under NRL support for 2018 to 2019. This was the first time that *Campylobacter* enumeration was included, as this is a new EU Process Hygiene Criteria. Overall, results were satisfactory, although there appears to be a decrease of OCLs achieving the 70% threshold, which the NRL will monitor. The NRL participated in 12 EURL PTs and received or indicated satisfactory performance for all. A practical PCR workshop, with STEC focus was organised by the NRL to support OCLs to implement PCR and STEC testing to their laboratories; feedback was very good for both events. A further Skype workshop is planned to address the implementation of the ISO:17025;2017 for UK OCLs.

Details of the proposed NRL activities for 2019 to 2020 and a timeline to achieve these complete this report.

Abbreviation list

AFBI	Agri-Food & Biosciences Institute
APHA	Animal and Plant Health Agency
AR	Antimicrobial resistance
BSI	British Standards Institute
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CEN	European Committee for Standardization
cgMLST	core genome Multi-Locus Sequence Type
CPS	Coagulase positive Staphylococci
DEFRA	Department for Environment, Food and Rural Affairs
ECDC	European Centre for Disease Prevention and Control
EFL	European Food Microbiology Legislation
EFSA	European Food Safety Authority
EPIS	Epidemic Intelligence Information System
EQA	External Quality Assurance
ESBL	Extended-spectrum Beta-Lactamases
ESM	European Screening Method
EURL	European Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FBO	Food Business Operator
FEPTU	Food and Environmental Proficiency Testing Unit
FSA	Food Standards Agency
FW&E	Food, Water and Environment
ISO	International Standards Organisation
MS	Member State
NRL	National Reference Laboratory
OCL	Official Control Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field Gel Electrophoresis
PHE	Public Health England
PT	Proficiency Test
RTE	Ready-to-Eat
SOP	Standard Operating Procedure
ST	Sequence Type
STEC	Shiga-toxin producing <i>E. coli</i>
WG	Working group
WGS	Whole Genome Sequencing
WHO	World Health Organisation

Introduction

The UK's Competent Authority, Food Standards Agency (FSA) has a contract with Public Health England (PHE) to provide the service of UK's NRL for food microbiology for since 2011. The NRL is responsible for the following work areas as defined in Regulation (EU) 2017/625 (previously (EU) 882/2004): *Listeria monocytogenes*, coagulase positive staphylococci, *Escherichia coli* (incl. STEC), *Campylobacter*, *Salmonella* and AMR. Public Health England has recently been awarded the NRL service to continue these activities until March 2023.

This report details the secretariat services, advice and representation within the UK/EU, production of documents, coordinating and participating in audits, ring trials and European Reference Laboratories (EURLs) initiatives and communication of results and data between April 2018 and March 2019. Table 1 list the NRL core functions and the activities are described in this annual report.

Table 1. PHE NRL Core Functions, April 2018 to March 2019

Core Function	Description
1	<i>Secretariat services</i>
1.a	Disseminate information/advice supplied by the EURLs to FSA, OCLs and other UK laboratories in a timely and effective manner
1.a	Produce and circulate quarterly newsletters to FSA, OCLs and other UK laboratories
1.b	Co-ordinate the OCL User Day to update UK OCLs and other relevant UK laboratories to the NRL core functions
1.b	Review content of the UK Food Examiner Register
1.b	Continue liaison meetings and produce a protocol for working together with APHA for AR, <i>Campylobacter</i> and <i>Salmonella</i>
1.d	Provide regular updates to the FSA on NRL activities by producing monthly reports and meet on a quarterly basis
1.d	Produce and submit annual report to the FSA on NRL activities for 2018 – 2019
1.e	Maintain and update the NRL web content on the PHE website
2	<i>Advice and representation within the UK/EU</i>
2.a	Provide impartial expert advice to FSA, OCLs and other UK laboratories, upon request
2.b	Represent the UK at relevant EURL meetings; consult FSA prior to meetings and submit an internal report after attendance of meetings

2.c	Attend training workshop at the STEC EURL for 'STEC identification and typing from food' (organised by EURL, Istituto Superiore di Sanità, Rome)
2.e	Keep abreast of methodology developments and advise FSA and OCLs (eg, workflow and Service Level Agreement for CPS toxin testing)
2.g	Participate in the BSI AW9 microbiology committee
2.g	Participate in Working Group to revise the ISO/TS 13136:2012 (PCR detection of STEC)
3	<i>Production of standard operating procedures, codes of practice and guidance documents</i>
3.a	Update and expand food methods archive on NRL website
3.a	Prepare a guidance document for OCLs and the FSA on the use and validation of alternative methods for testing Official Controls
3.a	Produce a poor performance protocol for OCL participation in the EFL proficiency test scheme
3.a	Perform gap analyses of ISOs from the EU Mandate 381 and related UK SOPs and update accordingly
4	<i>Compliance assessment via audits and ring trials</i>
4.a	Ensure consistency and quality of testing applied by UK OCLs and support where necessary
4.b	Liaise with FEPTU and monitor OCL's comparative testing performance and assist OCLs in the implementation of corrective measures
4.d	Participate as UK-NRL in ring trials including method comparison or validation studies and other initiatives organised by the EURL (on-going) and report to FSA
4.e	Organise a Skype/classroom-based workshop for UK OCLs
4.e	Organise a PCR workshop (with STEC detection focus) for UK OCLs
5	<i>Co-ordination within the UK of EURL initiatives</i>
5.a	Support the food aspect of the EU-wide AR monitoring (Decision 2013/652/EU), liaising with FSA, OCLs relevant Reference Laboratories and APHA. Liaise with APHA, audit and review strategy for harmonization of existing antimicrobial resistance testing
5.a	Provide information regarding Whole Genome Sequencing when requested from the EURLs, and participate in any related workshops, training and guidance documentation, where necessary
6	<i>Communication of results and data use</i>

Core Function One: Secretariat services

Dissemination of information from the EURLs

The NRL receive information from the 6 EURLs concerning new reports, outbreaks and other related topics regularly. Information is then cascaded to the appropriate stakeholder(s) (e.g., OCLs, FSA, Scottish Reference Laboratories), with any additional information or advice on further steps to be taken. The EURLs also send questionnaires and surveys to the NRLs regarding NRL and/or country-wide practices. These communications are described below by work activity with links to the EURLs' websites; information concerning meetings, training, proficiency tests (PTs) and ISOs are incorporated in the relevant sections of this report. Where available, the EURLs' work programmes can be found in the Annex.

Listeria monocytogenes

Website: <https://eurl-listeria.anses.fr/en/minisite/listeria-monocytogenes/eurl-listeria-monocytogenes>

The EURL sent a protocol for the DNA extraction from *L. monocytogenes* in April 2018 and this was sent on to PHE colleagues.

In July 2018, the EURL forwarded an EFSA-ECDC Rapid Outbreak Assessment and technical reports from the investigation into a multi-country outbreak of *Listeria monocytogenes* ST6 linked to frozen sweetcorn; these were cascaded to the OCLs and PHE colleagues.

The EURL sent an invite in November 2018 to a shelf-life studies workshop, organised under the Better Training Safer Food initiative of the European Commission. This workshop was held in Brussels on 27-28 March 2019 and the invitation was forwarded to colleagues from Campden BRI.

In December 2018, the EURL circulated several news links from their own website, describing recent EURL news and activities. These included relevant working group meetings, the ECDC-EFSA joint zoonotic report for 2017 data, and training for challenge testing. This email was cascaded to relevant stakeholders.

The EURL cascaded a report in February 2019 which details the use of WGS data to identify targets from each major *L. monocytogenes* cgMLST clonal complex, which could then be used to predict and screen those cgMLST clonal complexes. The EURL recommended that in the absence of WGS capability, a real-time PCR assay using

these targets could be used to align with WGS-generated cgMLST. This report was sent to relevant PHE colleagues.

In February 2019, the EURL announced that the retail temperatures in Table 3 of the Technical Guidance for shelf-life testing has been amended and accepted by the Standing Committee of the Food Chain & Animal Health/Section 'Biological safety of the food chain'. These temperatures have been lowered from 12°C to 7°C, to reflect recent EU data.

The EURL sent a report in March 2019 on the EURL's assistance to the Czech Republic in the investigation of a plant contamination with *L. monocytogenes*, using WGS data which linked human cases to strains found in the processing plant.

In March 2019, the EURL forwarded their method for molecular serotyping for *L. monocytogenes* to the NRLs, which mentions performance characteristics and additional information on the reagents and material used. This was cascaded to relevant PHE colleagues.

The EURL circulated a further news update in March 2019, including relevant working group meetings, an announcement of a joint scientific conference on food pathogens and WGS, sharing WGS skills with the Netherlands and the amendment of the microbiological criteria (EC) 2073/2005. This email was cascaded to relevant stakeholders.

Between April 2018 and March 2019, there have been 9 EURL correspondences concerning 8 Epidemic Intelligence Information System (EPIS) enquiries of *L. monocytogenes* clusters. The NRL logs these and contacts the relevant staff in PHE, as the EURL are requesting information on any related isolates from non-human derivation. PHE staff then looks in the UK database to identify any closely related strains relating to the cluster concerned. The NRL then responds to the EURL of any information from PHE colleagues concerning the enquiry and forwards the information to FSA. Examples include EPIS 461, which was related to the ST6 sweetcorn incident, in which the UK experienced both clinical and food isolates, and sent 2 clinical isolates to the EURL for PFGE typing, and EPIS 509, where MLST CC9 was causing a multi-country outbreak of listeriosis and is associated with gravid salmon; the UK observed no non-human isolates in their database at the time of their response.

Coagulase positive staphylococci

Website: <https://eurl-staphylococci.anses.fr/en/minisite/staphylococci/eurl-coagulase-positive-staphylococci>

In December 2018, the EURL circulated several news links from their own website, describing recent EURL news and activities. These included relevant working group meetings, the ECDC-EFSA joint zoonotic report for 2017 data, and training for detection of staphylococcal toxin genes by multiplex real-time PCR. This email was cascaded to relevant stakeholders.

The EURL sent a further email news update in February 2019, which highlighted the change from the European Screening Method for enterotoxin detection being cited in the EU microbiological criteria (EC) 2073/2005, to the ISO 19020:2017 reference method. Other news included relevant working group meetings and events to train the detection of staphylococcal toxin.

In February 2019, the EURL cascaded information concerning ELISA-based quantitative values for the certified reference material on SEA in cheese powder to NRLs. This information was forwarded to relevant colleagues.

Escherichia coli (including STEC)

Website: <http://old.iss.it/vtec/>

In April 2018, the EURL sent a new laboratory method for identification of STEC serogroups mainly associated with human infections by Real-Time PCR amplification of O-associated genes (EURL-VTEC_Method_11_Rev 0), which was cascaded to PHE *E. coli* experts (see Annex).

The EURL has cascaded specific outbreak information, meetings and reports to the NRLs, including:

- various updates of a multistate outbreak of STEC O157 in the US linked to romaine lettuce
- a US EHEC O157 outbreak from last year in Utah, thought to be transmitted from animal manure
- 2 clusters of *E. coli* EHEC from 2 different sources (unpasteurised cow's milk and a daycare centre associated with goat exposure), occurring in Tennessee, USA
- news from the Health Service Executive on increased numbers of *E. coli* EHEC infection in Ireland
- a joint JEMRA FAO/WHO report on Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring

- cases of *E. coli* EHEC in Oklahoma, USA linked to possible exposure to recreational water
- publication of 'Emerging multidrug-resistant hybrid pathotype Shiga toxin-producing *E. coli* O80 and related strains of clonal complex 165, Europe.' Cointe A et al, *EID*, 2018, vol 24
- an outbreak in France caused by an O26 strain, where unpasteurised cheese is implicated
- invitation to a Next Generation Sequencing workshop for STEC and Mycobacteria, hosted by Austrian-NRL AGES, 20-22 March 2019, Vienna
- the launch of the updated One Health European Joint Programme website, which includes an events and announcement page for all the associated projects;
<https://onehealthjp.eu/>

Campylobacter

Website: <http://www.sva.se/en/service-and-products/eurl-campylobacter>

In September 2018, the EURL forwarded a 'save the date' announcement for the next *Campylobacter* CHRO meeting in Belfast 8-12 September 2019

Salmonella

Website: <https://www.eurlsalmonella.eu/>

Four newsletters were received by email link, informing NRLs of the EURL activities, including proficiency tests and workshop preparation and a literature search of relevant *Salmonella* scientific papers. These were forwarded to the FSA, the OCLs and other relevant laboratories in the UK. In brief these were:

- the June 2019 edition included news about an amendment to ISO 6579-1, Annex D for *S. Typhi* and *S. Paratyphi*, details of their organisation of proficiency tests, their work programme for 2018 and activity report for 2017
- the October edition reported proficiency test updates and related discussions from the ISO/TC34/SC9 and CEN/TC275/WG6 annual meetings
- the December edition included proficiency test organisation, and the publication of the validation of the revised ISO 6579-1:2017
- the March 2018 edition covered the organisation of proficiency tests, a multi-country outbreak of *Salmonella* Coeln and the amendment of the microbiological criteria (EC) 2073/2005

The EURL newsletters can be found in the Annex.

Between April 2018 and March 2019, there have been 6 EURL correspondences concerning 4 Epidemic Intelligence Information System (EPIS) and 2 NRL enquiries of *Salmonella* clusters. The NRL logs these and contacts the relevant staff in PHE, as the EURL are requesting information on any related isolates from non-human derivation. PHE staff then looks in the UK database to identify any closely related strains relating to the cluster concerned. The NRL then responds to the EURL of any information from PHE colleagues concerning the enquiry and forwards the information to FSA. Examples include EPIS 472, where the Czech Republic experienced a rise in *S. Bareilly* cases and the UK sent data for 200 isolates to the EURL for comparison and EPIS 526, in which *S. Coeln* were causing multi-country cases; the UK observed no non-human isolates in their database at the time of their response.

Antimicrobial resistance

Website: <https://www.eurl-ar.eu/>

The EURL sent a notification in April 2018 of a commercially available microbroth plate to test for colistin resistance and a recommended microtitre plate template to use with them. This was forwarded to relevant colleagues.

In July 2018, the EURL launched a second round of their E-learning course on metagenomics and how they analyse the WGS data from complex samples, which was cascaded to relevant PHE colleagues.

The EURL invited NRLs to participate in uploading WGS data to a restricted hub, the European Nucleotide Archive in October 2018. This was voluntary and required to sign up to a code of conduct to ensure data are protected from misuse. The email was forwarded to PHE colleagues for interest.

The annual EURL newsletter was sent to all NRLs in December 2018 (see Annex), which focused on identifying sporadic occurrence of ertapenem resistance of *E. coli*, Carbapenem-resistant *Enterobacteriaceae* from imported shrimps, and the EURL-AR training course on WGS held in September 2018.

The EURL also sent specific documents, reports or notifications from other European bodies and were forwarded to relevant colleagues and stakeholders. These were:

- a review on 2 decades of the National Antimicrobial Resistance Monitoring System
- a progress report on the implementation of the 2017 AMR Action Plan and a webpage on AMR national Actions Plans and Strategies
- the World Health Organisation (WHO) online course 'Antimicrobial Stewardship online course: a competency-based approach', which over 10,000 people have enrolled within the first 3 months

- a tripartite Memorandum of Understanding between Food and Agriculture Organization of the United Nations (FAO), OIE (World Organisation for Animal Health) and WHO, agreeing to strengthen their long-standing partnership, with a strong focus on tackling antimicrobial resistance
- a publication describing an emerging phenicol, oxazolidinone and tetracycline resistance gene (Antonelli et al, JAC 2018)
- a WHO/FAO Summary report of Expert Meeting on Foodborne Antimicrobial Resistance: Role of the Environment, Crops and Biocides
- a joint global database by WHO, OIE and FAO, detailing country progress on the global action plan on antimicrobial resistance
- the eighth report of the European Surveillance of Veterinary Antimicrobial Consumption
- a request from VetCAST for antimicrobial data from isolates from cats, dogs and pigs (not relevant for the food microbiology NRL)
- the UK One Health Report - Joint report on antibiotic use and antimicrobial resistance, 2013-2017
- European Medicines Agency's Antimicrobial Advice ad hoc Expert Group draft scientific advice on the categorisation of antimicrobials
- a link to a WHO webinar entitled 'Detection and reporting of colistin resistance'
- an invitation to a WHO GLASS webinar, 'Global Sewage Surveillance Project'
- the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017 from EFSA and ECDC

Parallel correspondence from various EURLs

There are some horizontal activities and regulations that some or all the EURLs are involved with; consequently, the UK NRL receives the same information from different EURLs. Those that were received between April 2018 and March 2019 and were cascaded to relevant stakeholders are:

- a draft amendment to EC Regulation 2073/2005, which was released for public consultation, and subsequently released as EC Regulation 2019/229
- the publication of 2 EU reports concerning the EU-wide WGS survey for food and waterborne pathogens and the final report of the EU project 'Establishing Next Generation sequencing Ability for Genomic analysis in Europe (ENGAGE)'
- a draft document entitled 'Guidance Document for the organisation of Proficiency Tests by NRLs for national networks, including partial outsourcing guidance' was sent to the NRLs for comment. The UK NRL submitted a single document submission to all relevant EURLs.

Related to Core Function(s): 1.a, 1.c, 2.d, 2.e, 2.f, 4.c, 5.a

Production of NRL quarterly newsletters

The NRL has produced quarterly newsletters since 2016, to notify OCLs and other stakeholders of NRL activities and areas that would affect them, such as the new Official Control Regulations (EU) 2017/625. These newsletters hope to maintain a regular harmonised approach of disseminating information. A brief description of the newsletters' content, available in the Annex, is:

- the 2018 OCL User Day, the OCL results from the *Campylobacter* PT, news from the 4 spring EURL meetings, and an ISO method update - June 2018
- OCL results from the 2017-18 EFL PT scheme and a document update - September 2018
- news from the 2 autumn EURL meetings, EU-Exit impact on NRL activities, a document update and upcoming events - January 2019
- a report on the OCL PCR workshop, EU-Exit, an ISO standards update and amendments to the microbiological criteria (EC) 2073/2005 - March 2019

Related to Core Function(s): 1.a, 2.d, 2.e, 2.f

Co-ordination of the 2018 OCL User Day

On 11 June 2018, the sixth Official Control Laboratories User Day was held at PHE Colindale and 37 colleagues attended from 10 OCLs and other key laboratories including the PHE's Food and Environmental Proficiency Testing Unit (FEPTU), the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit and the Gastrointestinal Bacteria Reference Unit, the Animal and Plant Health Agency (APHA), the FSA, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) and Campden BRI.



As well as updates from the EU Reference Laboratories (EURLs), topics including antimicrobial testing in foods, STEC detection by the ISO 13136 revision, OCL performance for *Campylobacter* testing, and the recent outbreak of listeriosis related to frozen sweetcorn, were presented. (see Annex).

Feedback from those attended was overall very good to excellent, and topics to cover for next year were suggested, which the NRL will endeavour to include. All the slide presentations are available upon request.

Related to Core Function(s): 1.a, 1.b, 1.c, 2.a, 2.d, 2.e, 2.f

Review content of the UK Food Examiner register

The NRL has established and maintained a Food Examiner register since 2014 to assist FSA to rapidly contact the appropriate local support from the OCLs if they receive enquiries concerning microbiological testing/investigation. The register was reviewed in 2018.

Related to Core Function(s): 1.b, 4.a

Liaise with APHA regarding mutual NRL activities (*Campylobacter*, *Salmonella* and antimicrobial resistance)

Since 2013, liaison meetings between PHE and APHA have taken place, as both Agencies are designated as NRLs for *Salmonella*, *Campylobacter* and antimicrobial resistance (AR) by their respective Competent Authorities, FSA and Department for Environment, Food and Rural Affairs (DEFRA). These meetings allow transparency between the 2 NRLs and to ensure there is co-ordination of activities. Since the EURLs will only financially support a single NRL per Member State at annual workshops, training events and participation in ring trials, PHE and APHA agreed that EURL funding would be allocated on an alternating basis. However, if activities of one organisation take precedence, eg that APHA undertakes the current statutory AR testing in the food chain across the EU, then that NRL will take priority for EURL funding.

PHE organised 2 meetings with APHA in July 2018 and January 2019, via teleconference. The Agri-Food & Biosciences Institute (AFBI) also participated, as they are the Northern Ireland Reference Laboratory for *Salmonella* and have direct communication with some of the EURLs. Discussion topics included the new Official Control Regulation 2017/625, annual NRL work programmes, and the agreement on EURL-funded activities. A table of NRL activities 2018 – 19 were drafted, circulated and agreed.

Related to Core Function(s): 1.b, 4.c, 5.a

Provide regular updates to Food Standards Agency

NRL representatives met with FSA quarterly (14/06/2018, 11/09/2018, 13/12/18, 11/03/19) to discuss progress made, difficulties met, and future or new activities (see Annex for minutes). In addition, monthly reports listing NRL activities have been submitted electronically to the FSA (see Annex).

Related to Core Function: 1.d

NRL Web Content

The NRL web page is on the Public Health England section of the .gov.uk website. The web page holds NRL annual reports since 2013, 8 standard methods, a public health management guidance, and reports of the 2016 and 2013 OCL audits. There is also general information about the NRL, expert witness information, and contact details. There are future plans to expand the NRL web presence by creating additional pages for each of the activities; *Listeria monocytogenes*, coagulase positive staphylococci, *Escherichia coli* (incl. STEC), *Campylobacter*, *Salmonella* and antimicrobial resistance, and to review, update and add to the standard methods.

The web-site address is <https://www.gov.uk/government/collections/uk-national-reference-laboratory-for-food-microbiology> . For ease of access, OCLs and other stakeholders are advised to use a search engine and type 'fwe nrl' or 'food NRL', as the NRL web page is normally the top hit.

Related to Core Function(s): 1.a, 1.b, 1.e, 3.a

Core Function 2: Advice and representation within the UK/EU

Provide impartial advice to FSA, OCLs and other UK laboratories

The NRL are increasingly receiving more requests for advice from small business organisations to European institutes.

General

General requests received between April 2018 and March 2019 were:

- a query from a hospital for fungal testing in a food sample; this was forwarded to the PHE mycology reference laboratory, who were happy to accept the sample
- a school requesting a tour around a FW&E laboratory
- advice sought from Campden BRI on measuring risk of *Clostridium botulinum* in challenge testing, growth of organism or toxin detection; NRL forwarded query to botulinum experts
- a query from commercial laboratory in Netherlands, for ISO-based proficiency schemes for infant formulae and cereal products, and if schemes can be provided to other countries; NRL sent FEPTU's contact details
- an enquiry from FSA seeking advice on differences of aerobic colony counts performed on raw milk samples at 2 different laboratories (dairy OCL and FBO laboratory); NRL scientists and FW&E laboratory lead advised in accordance to the information supplied. Further advice sought from FSA regarding testing methods for examining raw drinking milk; NRL supplied information
- an OCL enquiry with regards to the Role of Food Examiner in witness of fact; the NRL Food Examiner advised
- an FSA enquiry about environmental swabbing in slaughterhouses and if there are existing ISO standards to carry out such work; the NRL sent ISO 18593 (surface sampling method), an EFSA report sampling from breeding pigs and information of other relevant ISO Standards
- a request from Campden BRI for temperature data of domestic fridges; the NRL replied that PHE does not collect this data routinely and forwarded Campden BRI to relevant EFSA reports and EURL data
- an enquiry from member of public regarding suspected poisoning from consuming supermarket pancakes; NRL advised to contact their Local Authority and speak to an Environmental Health Practitioner

- an OCL enquiry regarding changes to ISO 11133 – Performance testing of culture media; NRL responded with the chronology of the ISO revisions and related amendments
- advice requested about using the QIASymphony to extract DNA for WGS from the Netherlands; liaised with PHE experts and replied with advice
- an FSA enquiry regarding a CODEX guidance of histamine testing in fish; NRL referred the FSA to AFBI and Fera Science Ltd, as PHE does not test for histamine in food
- an enquiry from FSA regarding EFSA data collection and reporting systems used in the UK; NRL replied with England reporting systems
- an email from a TV director requesting information on testing food pathogens for a documentary; NRL forwarded email to PHE Communications
- a request from a commercial company to carry out testing of a prototype near-sampling machine for microbiological analysis of environmental samples; NRL replied that FW&E laboratories are unsuitable for this testing and a further teleconference was held to advise this company further
- an OCL enquiry regarding methodology of *Acinetobacter* detection in water; NRL forwarded to PHE colleague who advised the OCL
- various enquiries from individuals wanting work experience and/or placements in food and reference laboratories; these were passed on to the PHE Training Manager
- a request for microbiological testing of moringa powder for total bacterial counts; *Clostridium botulinum* testing in cured meats from a UK Food Business Operator (FBO); legionella testing from South West Water; certifying the import of South African foods; microbiological testing of sprouts from an FBO; environmental swab testing from a medicinal manufacturer; bacterial and nutritional testing in sauces from an FBO
- a draft strategy document from the Public Health Microbiology Group of the Scottish Health Protection Network
- an OCL request for advice on producing standard curves for quantitative PCR assay (for *Bifidobacterium*); the NRL advised that there is no specific ISO for *Bifidobacterium* quantification in food by PCR, but general PCR ISOs do exist; it is difficult to source DNA for this bacterium as reference material

There was a request for a site visit from a Chinese delegate of water engineers to learn methodologies for detection of chemical pollutants in river water at FW&E; the NRL advised the Drinking Water Inspectorate of DEFRA or the Chemical Hazards Unit in PHE would be the suitable people to contact for this visit.

The UK NRL for Food Microbiology attended 4 multi-organisation meetings to address how the NRL activities would be affected by a no deal EU Exit. These were between August 2018 and March 2019, chaired by Defra and attended by several other UK NRLs and FSA. A related meeting was held to discuss contingency plans in case of no-deal

EU Exit. An email from CEFAS was received in March 2019, regarding the NRL's EU Exit status with the *Salmonella* and *E. coli* EURLs, as CEFAS may also be affected; the NRL advised of the situation.

In September 2018, the FSA forwarded the proposed approach to retain EU law for Food and Feed Safety and Hygiene, Official Controls and the Microbiological Criteria. The NRL submitted comments to FSA. In February 2019, draft Statutory Instrument (SI) documents were forwarded by the FSA; UK SI 2019 No. 665 was made legal on 22nd March 2019 and will come into force on exit day (see Annex).

Fera Science Limited contacted the UK NRL for Food Microbiology in October 2018 concerning the FSA Official Laboratories review, gathering information of laboratories performing Official Control work in accordance with EC Regulation 625/2017. The NRL responded with a list of OCLs plus results of the 2016 OCL audit for UK laboratory capacity and capability. The NRL also gave information to phase 2 of the review in February 2019, which is being organised by Ernst and Young. The NRL provided them with written advice and participated in a teleconference to clarify funding arrangements, allocation, sampling trends, workload, arrangements for crisis management and training.

Listeria

Requests about listeria received between April 2018 and March 2019 were:

- an OCL enquiry concerning formal samples and listeria testing and confirmation; NRL advised
- a product alert was received in March 2019 from Biomerieux regarding a product shortage of API *Listeria* kits; availability of this confirmation test may affect other laboratories and therefore this was cascaded to all OCLs

In May 2018, the EURL requested the NRLs for *L. monocytogenes* strains or sequence data from particular clonal complexes. The NRL sent this request to colleagues in PHE.

Coagulase positive staphylococci

The EURL sent an enquiry to the NRLs in April 2018 regarding the use of new technologies to characterise CPS used in the NRLs. The NRL replied, stating that PHE characterise >5000 CPS isolates, using a variety of techniques, including toxin gene detection using PCR, PFGE, spa typing, MALDI-TOF and WGS. In addition, the EURL requested volunteers to join a working group on the use of new technologies, which the NRL put forward a streptococcal typing PHE expert and was accepted.

An OCL enquiry was received regarding staphylococcal toxin detection in a dairy product; the NRL replied requesting further information to ascertain bacterial levels, nature of sampling and amount of remaining product. The OCL did not reply

Escherichia coli (including STEC)

In December 2018, EFSA emailed a questionnaire to support an EC mandate (00293) on the pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. Topics included methodology used, sampling strategy and submission of data. The NRL consulted UK STEC experts and completed the survey on-line.

Campylobacter

In May 2018, a former EURL expert requested information from the UK, analyses and experience with regards to the Process Hygiene Criteria on *Campylobacter* to deliver NRL experiences at the Better Training for Safer Food courses, an EU initiative. The UK NRL liaised with APHA and FSA and responded with the UK's experiences regarding sampling, testing and reporting of *Campylobacter*.

The EURL requested permission to use *Campylobacter* strains from a 2008 baseline study to use for validation studies of an annex to ISO 10272, describing PCR detection methods; the UK gave permission.

In September 2018, the EURL sent a survey to all the NRLs requesting information on methods used to detect *Campylobacter* in milk; the UK NRL submitted information

Antimicrobial resistance

In June 2018, the EURL circulated a questionnaire from EFSA on AR monitoring in MSs to all the NRLs, to inform the revision of EU Decision 2013/652/EU. Information gathered included isolating *Campylobacter* spp. monitoring of colistin resistance, further characterisation of ESBL/ampC/carbapenemase producers, and monitoring of MRSA in food-producing animals and food. As PHE shares NRL antimicrobial resistance responsibilities with APHA for the UK, the UK NRL liaised with APHA with information and APHA submitted the questionnaire on behalf of the UK.

Requests about antimicrobial resistance received between April 2018 and March 2019 were:

- an invitation from the EURL-AR on behalf of the GSS (Global Sewage Surveillance group) to participate in the second phase of slaughterhouse waste water collection;

the NRL forwarded to APHA who confirmed that this was for the attention of the VMD (Veterinary Medicines Directorate) for coordination

- a call to submit WGS data of unusual resistant phenotypes/genotypes to the Joint EURL-AR and CoVetLab database; NRL requested further information on data protection and code of conduct, which the EURL-AR duly resolved
- EUCAST (European Committee on Antimicrobial Susceptibility Testing) requested colistin MIC distributions to determine ECOFFs for *Acinetobacter*
- OCL enquiry concerning media suppliers for AR method; NRL advised

European Commission’s Microbiological Criteria Working Group

The NRL liaises with the FSA, when requested, to support FSA’s participation at the European Commission’s (EC) Microbiological Criteria Working Group. The NRL provided FSA information and advice on:

- A guidance document to evaluate the competence of laboratories implementing challenge tests and durability studies related to *Listeria monocytogenes* in RTE food
- categorisation of ready-to-eat food and consumption of non-RTE food as RTE
- a possible extension of staphylococcal enterotoxin detection to other categories than dairy products

Related to Core Function(s): 2.a, 2.d

Representation at relevant EURL meetings and prepare meeting reports

The UK NRL has sent at least one participant to attend the 6 EURL meetings for the time period of this report; *Listeria monocytogenes*, coagulase positive staphylococci (CPS), *Escherichia coli* (incl. STEC), *Campylobacter*, *Salmonella* and antimicrobial resistance (see Table 2). Where available, agendas for the meetings were forwarded to the FSA as they were received (see Annex); presentations from the UK were made at the *Listeria*, *Campylobacter* and *E.coli* meetings. Individual meeting reports were submitted to FSA after attending the meeting (see Annex).

Table 2. List of EURL meetings, April 2018 to March 2019

EURL Meeting	Date: From	Date: To	Location	EURL funded	Other attendees
Antimicrobial resistance	05/04/2018	06/04/2018	Kgs Lyngby, Denmark	Martin Day	Frieda Jorgensen
<i>Listeria monocytogenes</i>	10/04/2018	12/04/2018	Paris, France	Amisha Vibhakar	Corinne Amar
<i>Salmonella</i>	29/05/2018	30/05/2018	Uppsala, Sweden		Amisha Vibhakar

Coagulase positive staphylococci	30/05/2018	01/06/2018	Paris, France	Shona Neal	Heather Aird
<i>Campylobacter</i>	08/10/2018	10/10/2018	Uppsala, Sweden		Amisha Vibhakar Craig Swift
<i>E. coli</i>	18/10/2018	19/10/2018	Rome, Italy	Frieda Jorgensen	Shona Neal

Related to Core Function(s): 1.a, 2.b

Attend training workshop at the EURL

A wide variety of training courses have been offered by the EURLs, including molecular typing of STEC by PFGE, detection of staphylococcal enterotoxin genes by multiplex real-time PCR, and various WGS training from the *E. coli* and Antimicrobial Resistance EURLs. All EU Member States (MSs) and associated countries can show interest and attendance is decided on experience and need of that MS. However, the UK does not apply to every course, as the UK may not perform the specific technique (eg PFGE) or that the UK has previously attended the specific training.

In August 2018, the NRL received an invitation to attend 'Predictive Modelling for *Listeria monocytogenes* in foods' from the *Listeria* EURL. The UK registered a participant to attend the training in October 2018, but unfortunately the EURL cancelled the training and will hold it in 2019.

The STEC EURL invited applications in January 2018 to attend practical training for several aspects of STEC at the EURL in Rome, Italy. The UK NRL submitted one application in February 2018 for 'Detection and characterisation of STEC from food by ISO/TS 13136:2012 and characterisation of strains isolated'. The EURL offered a funded place and a UK representative attended this in May 2018 (see Annex for program). The PHE representative found the training useful, as it provided basic principles of the organism with a hands-on approach. They would recommend this EURL training to others for the background training in PCR and appreciate the difficulty in isolating STEC.

Related to Core Function(s): 2.c, 2.e

Participation in the BSI AW9 microbiology committee and other working groups

The BSI AW9 committee normally convene twice a year. However, for this 12-month period, there was only one meeting in May 2018, as the horizontal ISOs affected by the EU Mandate M/381 have largely been updated with performance parameters. A representative from the UK NRL for food microbiology attended this meeting and the status of all the related ISO standards were reviewed. The chair of the AW9 committee requested PHE/NRL to ask APHA to nominate a representative on this committee, as the microbiology methods are related to the food chain, including primary production;

the NRL contacted the APHA, who duly nominated someone to participate in the committee.

The NRL representative receives draft and final draft ISOs (DIS and FDIS) from the BSI AW9 portal throughout the year. Consequently, the NRL submitted comments for:

- ISO 22117 (Specific requirements and guidance for proficiency testing by interlaboratory comparison)
- ISO 15216-2 (detection of hepatitis A virus and norovirus in food using real-time RT-PCR)
- ISO 19036 (Uncertainty of Measurement)
- Parts 4 to 6 of the ISO 16140 series (Method validation of alternative methods)
- ISO 20976-1 (Challenge tests to study growth potential, lag time and maximum growth rate)
- ISO 6887-5 (Specific rules for the preparation of milk and milk products)

A representative of the UK NRL is a member of the CEN TAG18 expert working group for the revision of the ISO TS 13136 (PCR detection of shiga toxin-producing *Escherichia coli*) which met in October 2018. The 2 parts of the ISO was discussed, which included:

- the enrichment broth and incubation temperature changed to buffered peptone water and 41.5 °C
 - at least 2 selective media to be used as mandatory
- remove serogroup O174, as this is not in the top 20 serogroups in the EU

Two working groups associated with shelf life studies have been established by the *Listeria* EURL. The UK NRL has nominated a representative from Campden BRI, who have expertise in this subject. The first WG is to revise the storage temperature at different stages of the cold chain; after several WG meetings, a proposed temperature of 7 °C at manufacturer and retail was presented to the EC Standing Committee on Plants, Animals, Food and Feed and was unanimously adopted by EU Member States in February 2019. The other WG was to develop training tools for Competent Authorities to evaluate if laboratories are competent in shelf-life studies; 3 WG meetings were attended and 6 slide sets have been produced and are due to be completed in June 2019.

A further working group has been set up by the CPS EURL for the use of new technologies for rapid characterisation of coagulase positive staphylococci (CPS), where a representative of the UK NRL is a member. A teleconference was held in January to kick-off discussions and a further meeting is planned in June 2019.

Related to Core Function(s): 2.e, 2.f, 2.g

Core Function 3: Production of standard operating procedures, codes of practice and guidance documents

Update and expand food methods archive on NRL website

Nine Standard Methods and/or guidance are available on the NRL website at the time of writing (Table 3). These methods are based on PHE in-house methods and ISOs, and may assist OCLs to comply with the requirements of the EU Microbiological Criteria Regulations. Most of the relevant ISOs been revised under Mandate M/381, and the corresponding NRL methods are undergoing review and updates. Other relevant PHE Standard Methods have been identified that complement the NRL activities; these are also under the re-formatting process and will be archived on the NRL website. In addition, the PHE SOPs are available to OCLs upon request.

Table 3. List of Standard Methods archived on the NRL website, March 2019

Document No.	Title	Version No.
FNES8 [F12]	Enumeration of coagulase positive staphylococci (<i>Staphylococcus aureus</i> and other species)	4
FNES26 [F2]	Preparation of samples and dilutions, plating and sub-culture	1
FNES3 [F8]	Enumeration of β -glucuronidase positive <i>Escherichia coli</i> : Pour plate method	3
FNES22 [F19]	Detection and enumeration of <i>Listeria monocytogenes</i> and other <i>Listeria</i> species	4
FNES28 [F22]	Enumeration of β -glucuronidase positive <i>Escherichia coli</i> – most probable number technique	3
FNES16 [F13]	Detection of <i>Salmonella</i> species	4
FNES15 [F21]	Detection and enumeration of <i>Campylobacter</i> species	2
FNES4 [E1]	Detection and enumeration of bacteria in swabs and other environmental samples	4
FNES18 [Q4]	Guidance on Public Health response: involvement of PHE Food Water and Environmental Microbiology laboratory staff in the investigation of outbreaks of food or waterborne disease	3

Related to Core Function(s): 1.a, 1.e, 3.a, 4.a

Prepare specific guidance protocols for OCLs and the FSA

The NRL have been requested by the FSA to produce draft guidance for validating alternative methods in place of the reference method for the testing of the food-borne

organisms in food, feed and environmental samples. There has been a delay in the ISO/CEN revision of the relevant Standard (ISO 16140) and only 2 of the 6 parts have been published at the time of writing. In addition, it is the third, fourth and sixth part that mostly influences the guidance for FSA (Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory, Part 4: Protocol for single-laboratory (in-house) method validation and Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing). Pending the publication of the international standard, the NRL will finalise the guidance in 2019 to 2020.

A poor performance protocol is required if any OCLs generate repeated poor results from the European Food Microbiology Legislation (EFL) External Quality Assessment Scheme. However, there has been no consistent poor performance from the participant results (see next section).

Related to Core Function: 2.a, 2.d, 3.a, 4.a

Gap analyses of ISO Standards related to EU Mandate 381

The majority of the ISO Standards that were affected by the EU Mandate 381 have now been published. A gap analysis is performed by PHE, comparing the new and old ISO Standards alongside the current PHE method. Potential ‘gaps’ or ‘impacts’ are identified between the 2 versions of the ISO Standard, which may need to be addressed in the PHE Method. The PHE Food, Water and Environmental Microbiology Network Methods Group discuss these at bi-monthly meetings, which the NRL attends. The PHE Method is revised, if an impact is agreed, and further validation or verification is performed, where necessary. There was only one NRL-relevant PHE Method that have had an Impact Assessment performed against the related ISO Standard in this reporting year (Table 4).

Table 4. Gap analysis of a PHE Method, April 2018 – March 2019

PHE Method No.	PHE Method Title	Superseded ISO	Revised ISO
FNES8 [F12]	Enumeration of coagulase-positive staphylococci (<i>Staphylococcus aureus</i> and other species)	ISO 6888-1:1999 + A1:2003	ISO 6888-1:1999 + A2:2018

This impact assessment is available to FSA and the OCLs.

Related to Core Function: 2.a, 3.a

Core Function 4: Compliance assessment via audits and ring trials

OCL participation in the European Food Microbiology Legislation Proficiency Testing Scheme

In 2018-19 the NRL continued with its support to OCLs in participating in external quality assessment. Regulation (EC) 625/2017 stipulates the requirements of OCLs to take part in interlaboratory studies upon request by the National Reference Laboratory and for the NRL to coordinate and assess performance of such comparative testing.

All Official Control Laboratories were invited to register to the European Food Microbiology Legislation (EFL) External Quality Assessment Scheme. Provided by the PHE Food and Environmental Proficiency Testing Unit (FEPTU), this scheme enables the performance assessment on the identification, examination and interpretation of microbiological results of foods tested against legislative criteria in EU Regulation 2073/2005 (as amended). A total of 12 samples over 4 distributions based on food categories within the regulation are sent out each year with results being submitted on a web-based form. Further details can be found at this link:

www.gov.uk/government/collections/external-quality-assessment-eqa-and-proficiency-testing-pt-for-food-water-and-environmental-microbiology#european-food-microbiology-legislation-scheme

OCL participation in the EFL scheme allows the NRL to directly compare performance and act independently from the scheme organisers. All results are anonymised by the scheme providers and the identity of each individual laboratory is not disclosed to the NRL. However, the NRL does monitor performance of each individual laboratory, and if laboratories are experiencing difficulties they are invited to contact the NRL and seek assistance.

Thirteen out of 14 OCLs registered to participate in all 4 of the distributions in the 2018-19-year scheme and results overall were satisfactory. Laboratories continue to demonstrate excellence in their proficiency with presence/absence methods (one false-positive result was reported this year (for *L. monocytogenes* in dried milk powder), which has been seen only once before (for the same organism in pasteurised cheese)). Enumeration values from relevant examinations were good, with laboratories producing results well within the statistically acceptable limits and close to the participant's median. It was noted that one laboratory produced values outside the limits for acceptance for 2 consecutive distributions and at the time of writing the NRL were

liaising with FEPTU to investigate this laboratory and assist with difficulties they may be experiencing.

Table 5 summarises the samples for 2018-19 and the performance of OCLs that carried out the examinations. There was an increase in the number of laboratories producing one or more result below the 70% satisfactory threshold (7 laboratories, compared to 2 last year) but only 2 of these produced an unsatisfactory result on 2 or more occasions.

This year was the first time *Campylobacter* testing was included in the EFL scheme, since the enforcement of limits for this organism within the micro-criteria Regulation (EC) 2073/2005 (as amended) on 1 January 2018. A simulated sample of broiler carcasses (EFL 139) was sent out on 7 January 2019 and 10 laboratories correctly identified the food category within the revised legislation. Nine of these laboratories correctly named the test and the number of samples from a batch required for compliance. For microbiological testing, 5 laboratories performed the examination to produce the correct result and thus scored maximum marks.

EFL 139 also saw a failure of most laboratories identifying the need to serotype strains for *Salmonella* Typhimurium and *Salmonella* Enteritidis. The requirement to test for the absence of these organisms applies to broilers after chilling, as they will go on to be placed on the market. Only 2 laboratories identified the correct food category for this examination and one of these laboratories also correctly stated they would refer the strains for further testing. A gap in the knowledge of the legislative requirements for this criterion remains amongst OCLs since its introduction in 2011, as demonstrated from this sample and laboratories were reminded of the footnote to ensure compliance against this category.

An improvement was seen in the categorising of ice creams for *Listeria monocytogenes* where laboratories understood that this sample does not support the growth of this organism due to the frozen nature of the product. A dried milk powder sample was less easy to class since the current regulations are unclear as to how these foods fit into the different categories for *Listeria monocytogenes* testing when sampled at the food business's premises.

Table 5. Overview of performance of the 2018 – 19 European Food Microbiology Legislation Scheme

Sample code	Brief sample details	Required examination(s)	OCLs achieving >70% of the maximum possible score of 8 ¹
EFL133	Spicy tuna sushi roll containing uncooked tuna in a spicy chilli sauce covered in a nori sheet with sushi rice. Sampled before leaving the food business operator	<i>L. monocytogenes</i> detection	13/13

EFL134	Mascarpone cheese made using pasteurised milk, sampled before the food has left the business operator	<i>L. monocytogenes</i> detection <i>Escherichia coli</i> Coagulase-positive staphylococci	13/13 12/13 10/12
EFL135	Freshly cut fruit salad with orange juice sampled at market (shelf life of 3 days)	<i>L. monocytogenes</i> enumeration <i>Salmonella</i> spp.	11/12 12/12
EFL136	Carton of chilled pasteurised liquid egg whites drunk as a body building supplement with a shelf-life of 7 days. Sampled at the end of the manufacturing process	<i>L. monocytogenes</i> detection <i>Enterobacteriaceae</i>	11/11 13/13
EFL137	Lemon, garlic and herb salted butter made with unpasteurised milk at shelf-life	<i>L. monocytogenes</i> enumeration <i>Salmonella</i> spp.	12/12 13/13
EFL138	Cooked frozen seafood paella made with prawns, mussels, monkfish and squid at end of manufacture	<i>Escherichia coli</i> Coagulase-positive staphylococci	12/12 12/13
EFL139	Carcases of broilers sampled after chilling	<i>Salmonella</i> spp. <i>Salmonella</i> Typhimurium <i>Salmonella</i> Enteritidis <i>Campylobacter</i> spp.	9/10 0/0 ² 5/5 ³
EFL140	Ground lean pork mince sampled at the end of the manufacturing process	Aerobic Colony Count <i>Escherichia coli</i>	9/11 10/11
EFL141	Freshly made beef carpaggio, made with raw beef tenderloin, oil, lemon juice, salt and pepper. Product placed on the market during shelf-life	<i>L. monocytogenes</i> enumeration <i>Salmonella</i> spp.	10/11 11/11
EFL142	Home-made ice cream made with pasteurised lactose free milk and finely chopped mixed fruits tested at shelf-life	<i>L. monocytogenes</i> enumeration <i>Salmonella</i> spp.	9/12 10/12
EFL143	Dried skimmed milk powder sampled at the end of the manufacturing process	<i>L. monocytogenes</i> detection <i>Enterobacteriaceae</i> Coagulase-positive staphylococci	2/5 ⁴ 9/12 9/12
EFL144	Double cream made from thermised milk sampled at shelf-life	<i>L. monocytogenes</i> enumeration <i>Salmonella</i> spp.	9/12 9/11

¹Number of laboratories achieving >70% compared to the total laboratories participating in the examination. Those that did not return any data or did not examine samples were not included in this table

²Serotyping of strains to ensure the absence of *S. Typhimurium* and *S. Enteritidis* is necessary as stipulated in footnote 20 since samples will be placed on the market after chilling. Although no laboratory performed this examination, 2 laboratories correctly identified the food category and one of these laboratories also correctly stated the name of the examination, the number of samples from a batch required for compliance and the referral of colonies to the reference laboratory

³An additional 5 laboratories correctly identified the food category

⁴Three laboratories correctly identified the food category and 4 the name of the examination. The current regulations are unclear on how milk powder fits into the different categories for *Listeria monocytogenes* testing when sampled at the FBO's premises

Data from the European Food Microbiology Legislation Scheme will continue to be assessed for performance. Consolidated reports are provided by FEPTU and the NRL now has 5 years' worth of data available for comparison, trend analysis and to evaluate

lessons learnt. For example, although the results from a sample of ice cream showed an improvement in the understanding that this product does not support the growth of *Listeria monocytogenes*, 5 laboratories identified the wrong food category for *Salmonella* testing for the same sample, from the information given in the sample details. Sample descriptions covering the 4 food types prescribed in the scheme continue to provide opportunities to learn and challenge laboratories and to reflect the large variety of foods that a laboratory may encounter for testing. Laboratories are reminded of the scheme design and that decisions on appropriate testing should be based on fact, not assumptions and should refer to the FEPTU guide to scoring for more information.

The NRL has invited all OCLs to register to the above scheme for the 2019 – 20 distributions (see Annex). The scheme is unique in its provision of education on specific legislation and in addition to regulations making clear the obligations of laboratories that perform official control work to participate in such comparative schemes. Participation of OCLs will provide overall assurance of laboratory competence, identify areas of weakness and further training as well as support compliance with laboratory quality standards and accreditation. OCLs will also continue to have access to expert advice and support from FEPTU and/or the NRL.

Related to Core Function(s): 4.a, 4.b

Participate as UK-NRL in EURL ring trials and other initiatives (2018 to 2019)

The NRL is mandated to collaborate with and participate in inter-laboratory comparative tests as organised by the European Reference Laboratories in each of the areas that the NRL are responsible for. In 2018-19, the NRL has received 11 ring trial distributions from all 6 EURLs, that covers the work carried out by OCLs and the reference laboratories in the UK, and includes tests for detection, enumeration, typing and antimicrobial resistance. Table 6 lists these activities and a summary of performance.

The NRL participated in the EURL *Listeria* trial on challenge testing for the first time this year. Regulation (EC) 2073/2005 (as amended) sets out quantitative limits for *Listeria monocytogenes* of 100cfu/g for ready-to-eat foods that are able to support growth of this organism when placed on the market during its shelf life. Challenge testing is a method prescribed within the regulation that is used to check that foods can meet these limits throughout its shelf life. Three batches of liver mousse were sent from the EURL, where participants were instructed to test them over a period of 3 months and in accordance to the EURL *Listeria* technical guidance for conducting shelf-life studies on *L. monocytogenes* in RTE foods. The NRL does not routinely perform this test and requested the expertise of Campden BRI, who specialises in implementing challenge tests and assessing the growth potential of *Listeria* in foods, to perform the testing on behalf of the NRL, which the EURL accepted.

Another test that the NRL does not perform is the detection of staphylococcal enterotoxins in cheese. A subcontracted laboratory, that is also a designated NRL and part of the CPS EU network, was asked to participate in this PT on behalf of the UK. The EURL sent a UK-designated PT shipment to the subcontracted laboratory and processed the samples. The results were then sent to the UK NRL, which was then submitted to the EURL. A summary of the UK NRL's performance for both trials is in Table 6.

The EURL *E.coli* this year continued its trials on comparative testing for the detection and isolation of STEC in food and water. The reference method as prescribed in regulation stipulates the enrichment of samples in modified tryptone soy broth - mTSB (for samples with a high background organisms), or buffer peptone water (BPW), and incubation at 37°C. The Working Group assigned to revise the Standard (ISO TS 13136:2012) have observed that the use of mTSB as an enrichment broth appears to hamper the growth of certain STEC strains, and the use of BPW would be more appropriate. In addition, the Working Group reviewed some evidence that a higher incubation temperature would counteract the issue of containing background microflora when enriched with broth containing no supplements. Therefore, the EURL dispatched 2 trials (one using contaminated sprout samples and one of contaminated irrigation waters) that instructed the use of BPW for enrichment and incubation at 41.5°C, and results were compared to previous trials done in accordance to the referenced protocol (using mTSB and 37°C as the incubation temperature) on the same matrices. The results of these trials will be used to inform the revision and improvement of the reference method which is also mandated to become a full international standard.

The NRL did not participate in the proficiency tests (PT) for antimicrobial resistance testing for Enterococci, staphylococci and *E.coli* in 2018-19, as the UK NRL adopts a different method to that stipulated in EU legislation (an agar dilution method is performed for antibiotic sensitivity testing, whereas the EURL and the NRL network uses a broth dilution method). This difference may impact when comparing results and the EURL decided not to include the UK NRL for food microbiology in the analysis in previous years. In addition, the UK Food NRL is not performing the sampling and analysis required for the EU antimicrobial resistance monitoring Decision (2013/652/EU). APHA, as the UK animal and feed NRL, are performing this activity. The results of their participation in this trial, and others they are obliged to perform, are also listed in Table 6.

Related to Core Function(s): 1.a, 2.e, 4.c, 4.d

Table 6. NRL participation in EURL ring trials, March 2018 to February 2019

Month Received	Organism – Test ¹	Reference	Matrix/Pure culture	UK Recipient ²	Comments
March 2018	<i>Campylobacter</i> – enumeration and voluntary detection and species identification in food	PT21	Chicken skin	PHE and APHA	PHE – satisfactory performance (100%) APHA – acceptable performance (80%)
March 2018	<i>Campylobacter</i> detection and species identification in primary production sample	PT22	Chicken faecal swabs	APHA	Satisfactory performance for detection (94%) and identification (100%)
April 2018	STEC – detection and isolation from food	PT21	Sprouts	PHE	Satisfactory performance
April 2018	<i>Listeria monocytogenes</i> – challenge testing in foods	PT2	Liver mousse	NRL subcontracts test to external laboratory	Satisfactory performance
April 2018	CPS – enterotoxin detection in food	EILA/ANSES/LSAI/SBCL/2018/01	Cheese and ready-to-eat foods	NRL subcontracts test to external laboratory	Excluded from performance due to technical error but results indicate satisfactory performance compared with the intended results
May 2018	<i>Listeria monocytogenes</i> – typing	EILA/Anses LSAI/SEL/2018/05	Pure cultures	PHE	Satisfactory performance
May 2018	CPS – enumeration in food	EILA/Anses LSAI/SBCL/2018/03	Shelled cooked prawns	PHE	Excluded from performance due to deviations from the reference method; results indicate satisfactory performance for high level sample and unsatisfactory for low level sample
May 2018	<i>Listeria monocytogenes</i> detection in food	EILA/Anses LSAI/SEL/2018/01	Diced poultry meat	PHE	Excluded from performance due to deviation from the reference method; results indicate satisfactory performance compared to the intended result
May 2018	AMR – <i>E.coli</i> , Enterococci, staphylococci	24	Pure cultures	APHA	Satisfactory performance for <i>E. coli</i> and staphylococci; Enterococci – deviations in the labile antimicrobial tigecycline which are under investigation
Sept/Oct 2018	<i>Salmonella</i> detection in primary production sample		Boot sock	APHA	Satisfactory performance (100%)

October 2018	AMR – <i>Campylobacter</i> , <i>Salmonella</i> & matrix	25	Pure cultures	PHE & APHA	PHE – satisfactory performance (<i>Campylobacter</i> and <i>Salmonella</i>) APHA – satisfactory performance
November 2018	<i>Salmonella</i> – serotyping	23 rd	Pure cultures	PHE and APHA	PHE – 100% APHA – 100%
November 2018	STEC – detection and isolation in water	PT22	Irrigation water	PHE	Satisfactory performance
November 2018	STEC – typing of virulence genes and serogroups	PT23	Pure cultures	PHE	Serotype and <i>stx</i> type matched 100% with intended

¹ AMR = Antimicrobial resistance testing, STEC = Shiga toxin-producing *E. coli*, CPS = Coagulase positive staphylococci

² PHE = Public Health England, APHA = Animal and Plant Health Agency

Organise a Skype-based workshop on Implementation of ISO 17025:2017 for UK OCLs

The NRL will organise a Skype session for April 2019 concerning the implementation of the revised ISO 17025:2017 (see Annex for agenda). The UK Accreditation Service requires that all testing laboratories should transfer their accreditation to the new version of the Standard, and the FW&E PHE laboratories will share their experiences after gaining such accreditation. An email was circulated to all the OCLs on further details about this training session.

Related to Core Function(s): 2.a, 3.a, 4.e

Organisation of a practical PCR (with STEC detection focus) workshop for UK OCLs

Feedback from previous NRL events indicated that PCR training was required, as not all OCLs have implemented this method in their laboratories. As a result of this, many OCLs are unable to process samples to detect STEC using PCR, as defined in the Technical Standard ISO/TS 13136:2012 and enforced in the Microbiological Criteria (EC) No 2073/2005 (Category 1.29).

Therefore, on 6-7 February, the NRL organised a practical workshop on PCR detection, with a focus on STEC, for OCL colleagues. This was similar to a previous workshop that was held in 2013 when the criteria for STEC in sprouts was recently added to the EC Regulation No 2073/2005 (see Annex for Agenda). The workshop began with presentations on the principles of PCR and of detecting food pathogens using PCR, but it was predominantly focused on learning practical molecular diagnostic methods. There was a tour of a linear flow PCR suite, a demonstration of culture isolation after a presumptive STEC PCR positive, and advice on how to interpret PCR results.

Seven participants came from OCLs in England, Wales and Scotland, and there were a lot of fruitful discussions throughout the 2-day course. Feedback was good overall, with some delegates understanding and feeling more confident with using real-time PCR after the workshop. Future NRL events requested included *Campylobacter* enumeration, validation/verification of alternative methods and interpretation of EC 2073/2005.

Related to Core Function(s): 2.a, 3.a, 4.e

Core Function 5: Co-ordination within the UK of EURL initiatives

Support food aspect of the EU-wide AR monitoring (Decision 2013/652/EU)

Since 1 January 2015, fresh meat at retail have been sampled and tested for the above EU Decision in the UK by the APHA, who commenced the slaughter monitoring in the previous year. The NRL have been available for support and advice to the APHA and FSA. There has been continuation by APHA in sampling and testing the retail component of the EU harmonised survey for the FSA in the reporting year.

The EURL circulated a questionnaire from EFSA to all the NRLs about the revision of EU Decision 2013/652/EU. The UK NRL liaised with APHA for this, as described above in Core Function 2; Provide impartial advice to FSA, OCLs and other UK laboratories.

Additional information regarding APHA liaison and AR work can be found above in Core Function One; Liaise with APHA regarding mutual NRL activities.

Related to Core Function(s): 1.b, 2.a, 5.a

Participate in EURL activities relating to Whole Genome Sequencing

In 2017, the EC sent EFSA and ECDC a mandate to expand the molecular typing data collection to WGS data. Therefore, a working group formed by EFSA, ECDC and the EURLs focused on collating WGS capacity from the Member States, to assess the bioinformatic pipelines available, the requirements necessary to analyse WGS data, and the support needed to implement WGS into MSs (eg, training, methods).

As part of this work, a Whole Genome Sequencing (WGS) questionnaire was jointly prepared by the EURLs and was circulated by all 6 EURLs to their NRL networks, in April 2018. Information requested included technical details used in both wet and dry laboratories, participation in WGS proficiency tests and training needed to support further implementation and harmonisation of WGS in MSs. The NRL completed and submitted the questionnaires for all 6 areas within the deadline.

The *E. coli*, *Campylobacter*, *Salmonella* and AR EURLs all sent a similar survey in August and September 2018 requesting details of the bioinformatic pipelines used to those NRLs that have implemented WGS. As the pipelines and interpretation are slightly different for all the pathogens, these were checked by the relevant pathogen WGS specialists and surveys were sent to the individual EURLs by the NRL.

Related to core function(s): 1.b, 2.c, 5.a

Annex: Documents produced from NRL activities

Core Function One: Secretariat services	
<p>Dissemination of information from the EURLs <i>Related to Core Functions: 1.a, 1.c, 2.d, 2.e, 2.f, 4.c, 5.a</i></p> <p>Quarterly newsletters <i>Related to Core Functions: 1.a, 2.d, 2.e, 2.f</i></p> <p>Coordination of 2018 OCL User Day <i>Related to Core Functions: 1.a, 1.b, 1.c, 2.a, 2.d, 2.e, 2.f</i></p> <p>Provide regular updates to FSA <i>Related to Core Functions: 1.d</i></p>	<ul style="list-style-type: none"> • EURL Lm_2018 WP_v2 • EURL Lm_2019_2020_WP • EURL CPS_2018 WP_v2 • 2018 Kirsten Mooijman - Work programme EURL-Salmonella second half 2018, first half 2019; Discussion on general items; Closure • 475_t1-eurl-update-2019-ws • EU_RL_VTEC_Method_11_Rev_0 • EURL-Salmonella Newsletter June2018 • EURL-Salmonella Newsletter September 2018 • EURL-Salmonella Newsletter December 2018 • EURL-Salmonella Newsletter March 2019 • AR EURL 461_2018-12-newsletter-no12 • NRL_newsletter_Q1_2018_Final • NRL_newsletter_update_september_2018 • NRL_newsletter_Q3_2018_FINAL • NRL_newsletter_Q4_2019 (2) • User day 2018 agenda_FINAL • UK NRL Monthly Log_APRIL 2018_KL_AV_JMcL_Snedits (Final) • UK NRL Monthly Log_MAY 2018_Final • UK NRL Monthly Log_JUN 2018_Final • UK NRL Monthly Log_JULY2018_Final • UK NRL Monthly Log_AUG2018_FINAL • UK NRL Monthly Log_SEPT2018_Final • UK NRL Monthly Log_OCT2018_Final • UK NRL Monthly Log_NOV2018_FINAL • UK NRL Monthly Log_DEC2018_Final • UK NRL Monthly Log_JAN2019_FINAL • UK NRL Monthly Log_FEB2019_Final • UK NRL Monthly Log_MAR2019_AV_SN edits (FINAL) • FSA-NRL Minutes 14 June 2018_KL_CL comment (FINAL) • FSA-NRL Minutes 11Sept2018_KL_SN_JMcL_AVedits (Final1) • FSA-NRL Minutes 13Dec 2018_KL_SN_JMcL_edits (Draft2) • FSA-NRL Minutes 11Mar2019 VM edits (Draft 2)
Core Function 2: Advice and representation within the UK/EU	
<p>Provide impartial advice to FSA, OCLs and other UK laboratories <i>Related to Core Functions: 2.a, 2.d</i></p> <p>Representation at relevant EURL meetings and prepare meeting reports</p>	<ul style="list-style-type: none"> • ukxi_20190665_en_UK Official Control Regs • Agenda workshop EURL_AR 2018_draft_v12032018 • AMR Internal report of EURL meeting 2018 v2 • 460_eurl-ar-ws-2018-minutes-final • DraftAgendaLm2018 • Internal report of EURL meeting Lm 11-12 April_FINAL

<p><i>Related to Core Functions: 1.a, 2.b</i></p> <p>Attend training workshops at the EURL <i>Related to Core Functions: 2.c, 2.e</i></p>	<ul style="list-style-type: none"> • EURL Lm_report WS Lm 2018 • Programme of workshop 2018 • Indiv Report of Salmonella EURL meeting 2018 draft • Report 2018-0024 EURL-Salmonella workshop 2018 • AgendaCPS2018 • Internal report of CPS Workshop 2018_final • EURL CPS_report 2018 workshop • Programme for 13th Campy_workshop 8-10Oct2018 • Internal Report of 13th EURL Campylobacter Workshop 2018 FINAL • Report EURL-Campylobacter workshop 2018 • Agenda_Workshop_2018 • Internal report of EURL meeting_E.COLI 2018_FINAL • EURL_VTEC_Training_Program_ISOTS13136_Rev_3_17122018_
<p>Core Function 3: Production of standard operating procedures, codes of practice and guidance documents</p>	
<p>Update and expand food methods archive on NRL website <i>Related to Core Functions: 1.a, 1.e, 3.a, 4.a</i></p> <p>Gap analyses of ISO Standards <i>Related to Core Functions: 1.a, 3.a, 4.a</i></p>	<p>www.gov.uk/government/collections/uk-national-reference-laboratory-for-food-microbiology</p> <ul style="list-style-type: none"> • ISO 6888-1_1999+A2_2018 CPS - IA
<p>Core Function 4: Compliance assessment via audits and ring trials</p>	
<p>OCL participation in the European Food Microbiology Legislation Proficiency Testing Scheme <i>Related to Core Functions: 4.a, 4.b</i></p> <p>Organise a Skype-based workshop on implementation of IS 17025:2017 <i>Related to Core Function: 2.a, 3.a, 4.e</i></p> <p>Organise a practical PCR (with STEC focus) workshop <i>Related to Core Function: 2.a, 3.a, 4.e</i></p>	<ul style="list-style-type: none"> • 2019-20 EFL PT Registration form BLANK • ISO 17025 programme 25Apr19 - draft (004) • PCR 2019 Workshop Programme_FINAL

Proposed PHE NRL activities, April 2019 to March 2020

1 Core Function One: Secretariat services

- 1.a. Disseminate information/advice from international organisations to FSA, OCLs and other UK laboratories in a timely and effective manner.
- 1.a. Produce and circulate quarterly newsletters to FSA, OCLs and other UK laboratories.
- 1.a. Co-ordinate the OCL User Day to update UK OCLs and other relevant UK laboratories of the NRL core functions.
- 1.a. Assist in the dissemination and advice of EPIS and other alerts from the appropriate authority.
- 1.b. Update and perform an audit to gather information regarding the OCL's capabilities and requirements.
- 1.b. Review content of the UK Food Examiner Register.
- 1.b. Continue liaison meetings with APHA for AR, *Campylobacter* and *Salmonella*.
- 1.c. Liaise with FSA in matters arising from implementing the new Official Control Regulation, (EU) 2017/625.
- 1.d. Provide regular updates to the FSA on NRL activities by producing monthly reports and meet on a quarterly basis.
- 1.d. Produce and submit annual report to the FSA on NRL activities for 2019 – 2020.
- 1.e. Maintain and update the NRL web content on the PHE website.

2 Core Function 2: Advice and representation within the UK and internationally

- 2.a. Provide impartial expert advice to FSA, OCLs and other UK laboratories, upon request.
- 2.b. Represent the UK at relevant international meetings and working groups; consult FSA prior to meetings and submit an internal report after attendance of meetings.
- 2.c. Attend training workshops at international organisations, where relevant and after successful applications.
- 2.e. Keep abreast of methodology developments and advise FSA and OCLs (eg, workflow and Service Level Agreement for CPS toxin testing).

- 2.g. Participate in the BSI AW9 microbiology committee.
- 2.g. Participate in Working Group to revise the ISO/TS 13136:2012 (PCR detection of STEC).

3 Core Function 3: Production of standard operating procedures, codes of practice and guidance documents

- 3.a. Update and expand food methods archive on NRL website.
- 3.a. Prepare a guidance document for OCLs and the FSA on the use and validation of alternative methods for testing Official Controls.
- 3.a. Produce a poor performance protocol for OCL participation in the EFL proficiency test scheme.
- 3.a. Draft a manuscript for peer-review summarising 4 years of OCL performance of the EFL PT scheme.

4 Core Function 4: Compliance assessment via audits and ring trials

- 4.a. Ensure consistency and quality of testing approached applied by UK OCLs and support where necessary.
- 4.b. Liaise with FEPTU and monitor OCL's comparative testing performance and assist OCLs in the implementation of corrective measures.
- 4.b. Evaluate OCL's performance using trend analysis.
- 4.c. Coordinate the participation of OCLs in international method validation studies and other initiatives and report to FSA.
- 4.d. Participate as UK-NRL in proficiency tests and method validation studies organised by the EURL (where available) and report to FSA.
- 4.e. Organise a Skype/classroom-based workshop for UK OCLs on the implementation of ISO 17025:2017.
- 4.e. Organise a practical workshop for UK OCLs, dependent on the outcome of the audit (1.b) and OCL needs.

5 Core Function 5: Coordination within the UK of international initiatives

- 5.a. Support the food aspect of the EU-wide AR monitoring (Decision 2013/652/EU), liaising with FSA, OCLs, relevant Reference Laboratories and APHA.
- 5.a. Liaise with APHA, audit and review strategy for harmonization of existing antimicrobial resistance testing.

- 5.a Provide information regarding Whole Genome Sequencing when requested from the EURLs, and participate in any related workshops, training and guidance documentation, where necessary.

Proposed NRL activities for April 2019 to March 2020

Activities	Function	Core	0	1	2	3	4	5	6	7	8	9	10	11	12
			March 2019	April 2019	May	June	July	August	September	October	November	December	January 2020	February	March
Produce & circulate quarterly newsletter to FSA, OCLs & other labs	Disseminate information	1.a													
OCL User Day meeting at NRL, 18 th	Meeting	1.a													
Perform OCL Survey for capabilities and capacity	Secretariat	1.b													
Review content of UK Food Examiner register	Secretariat	1.b													
Continue liaison meetings with APHA <i>Salmonella</i> , <i>Campylobacter</i> and AMR NRLs	Secretariat	1.b													
Liaising with FSA matters implementing (EU)2017/625	Advice/communication	1.c													
Annual report to FSA	Coordination	1.d													
Meetings with FSA	Coordination	1.d													
Monthly reporting to FSA	Coordination	1.d													
Maintain and update NRL web content on PHE website	Website	1.e													
<i>Listeria</i> 13 th Workshop in Anses, Paris, 10-12 th	EURL Workshop	2.b													
Antimicrobial Resistance 13 th Workshop in Copenhagen, 25-26 th	EURL Workshop	2.b													
STEC detection and typing from food training, 13-17 th	VTEC EURL Training	2.c													
<i>Salmonella</i> Workshop in Uppsala, Sweden, 28-29 th	EURL Workshop	2.b													
Coagulase positive Staph 13 th Workshop in Anses, Paris, 26-28 th	EURL Workshop	2.b													
<i>Campylobacter</i> 14 th Workshop in Uppsala, Sweden, 7-8 th	EURL Workshop	2.b													
Organisation of <i>Campylobacter</i> PT training, 9 th	Campy EURL Training	2.c													
<i>E. coli</i> /STEC 14 th Workshop in Rome, 4-5 th	EURL Workshop	2.b													
Enum, detect, and species ID of <i>Campylobacter</i> spp training, 12-15 th	Campy EURL Training	2.c													
Agree the workflow and Service Level Agreement for CPS toxin testing with the Dutch NRL	Advice	2.e													
Participation in WG for revised ISO 13136 (STEC)	Advice & representation	2.g, 5.a													
Participation in BSI AW9 microbiology committee	Advice & representation	2.g													
Produce guidelines on alternative methods	Guidance document	3.a													
Produce poor performance protocol for OCL PT participation	PT document	3.a													
Produce OCL performance review of 4 years' participation of EFL scheme	Peer-reviewed manuscript	3.a, 4.b													

Activities	Function	Core	0	1	2	3	4	5	6	7	8	9	10	11	12
			0 March 2019	1 April 2019	2 May	3 June	4 July	5 August	6 September	7 October	8 November	9 December	10 January 2020	11 February	12 March
Revise and publish UK SOPs on website	Maintain SOPs	3.a													
Liaise with FEPTU & monitor OCL's testing of EFL scheme	UK PT	4.b													
Coordinate participation of OCLs in international method validation studies	International ring trials	4.c													
<i>Campylobacter</i> enumeration in chicken meat PT from EURL (PT23)	EURL PT	4.d													
<i>Campy</i> detect & species id in chicken meat PT from EURL (PT24)	EURL PT	4.d													
<i>Campylobacter</i> subtyping PT from EURL (PT25)	EURL PT	4.d													
<i>Listeria</i> enumeration in environmental samples from EURL	EURL PT	4.d													
<i>Salmonella</i> detection in flaxseeds PT from EURL	EURL PT	4.d													
STEC detection in sprouts from EURL (PT 21)	EURL PT	4.d													
Coag+ Staph toxin detection PT from EURL	EURL PT	4.d													
Coag+ Staph enumeration in food EQA from EURL	EURL PT	4.d													
AMR for <i>E. coli</i> , enterococci and staphylococci EQA from EURL	EURL EQA	4.d													
STEC detection in sprouts from EURL (PT 25)	EURL PT	4.d													
AMR <i>Salmonella</i> & <i>Campylobacter</i> EQA from EURL, TBC	EURL EQA	4.d													
<i>E. coli</i> & VTEC <i>vir</i> gene typing and WGS PT from EURL (PT-PFGE8;PT26;WGS3)	EURL EQA	4.d													
<i>Salmonella</i> typing EQA from EURL	EURL PT	4.d													
Organise practical workshop for UK OCLs (TBC)	Workshop	4.e													
Organise Skype workshop on implementation of ISO 17025:2017 for UK OCLs	Workshop	4.e													
Support food aspect of EU-wide AR monitoring (Decision 2013/652/EU)	EU monitoring	5.a													
Provide information regarding WGS processes to EURLs and other international organisations	EURL initiative	5.a													