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toute une vie en bonne santé

NRL-SALMONELLA BELGIUM

EURL- Salmonella Workshop 22-23 2022

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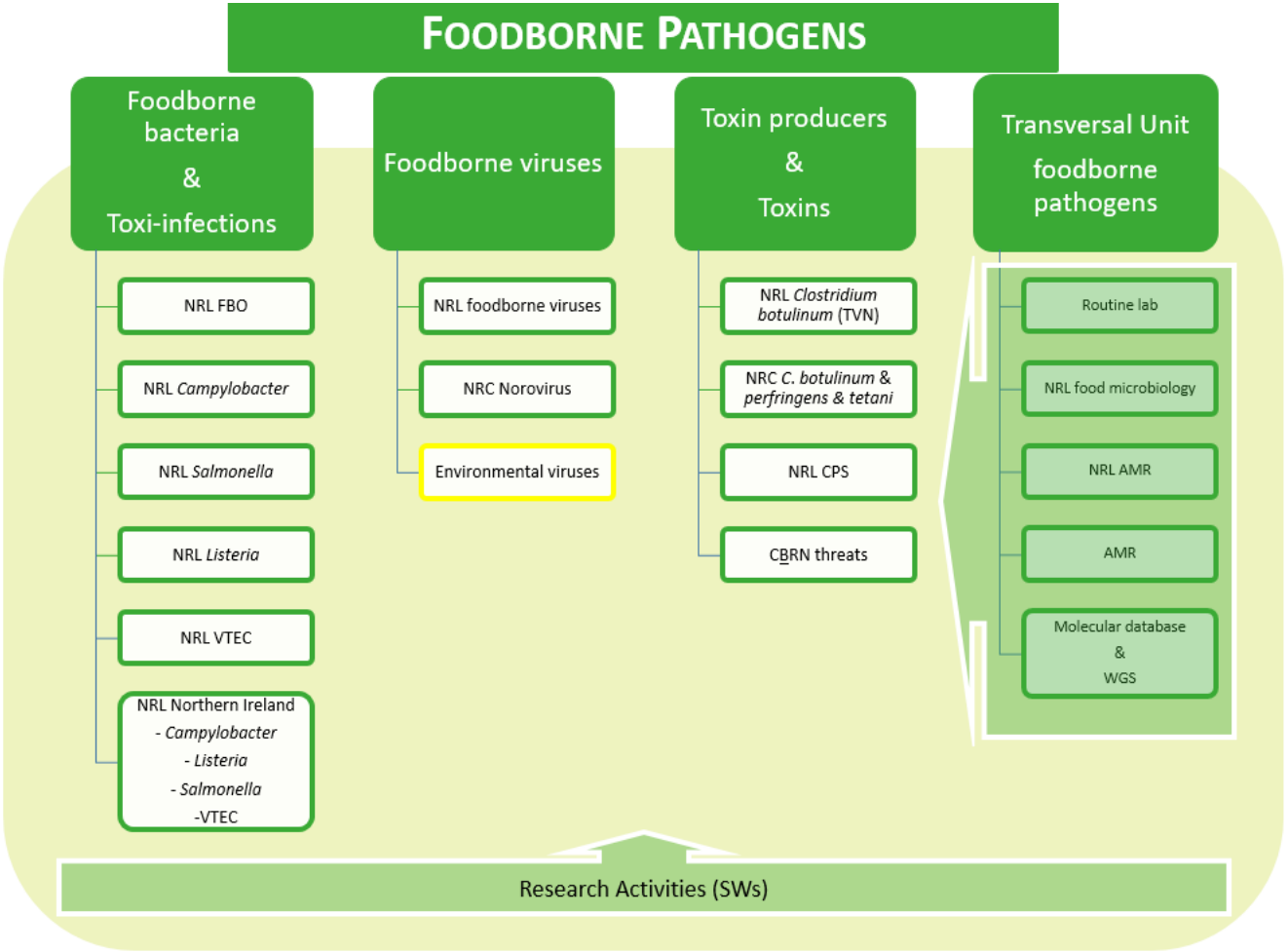
FoodBorne Pathogens-Sciensano



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Requirements/Duties for NRLs

L 95/80 EN Official Journal of the European Union 7.4.2017
http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0625&rid=3

- Analytical methods: Harmonizing and improving the methods of laboratory analysis
- PT: Organize inter-laboratory comparative testing or proficiency tests between official laboratories
- FBO outbreaks: Assist diagnosis of outbreaks of foodborne-*Salmonella* spp.
- Training: training courses for the staff of official laboratories designated
- Dissemination: Ensure the dissemination to the competent authorities and official laboratories of information that the European Union reference laboratory supplies

ISO 16140-3:2021 Method validation

Part 3: protocol for the verification of reference methods and validated alternative methods in a single laboratory.

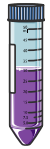
iQ-Check method for the detection of *Salmonella* spp. by qPCR
(AFNOR BRD 07/06-07/04)

To introduce a rapid screening method for *Salmonella* spp.,
mainly for foodborne outbreaks

Scope of validation: « Broad range of foods » (scope ≥ 5 food categories/ test portion 25g)
~~Primary production, environmental~~

Two steps for the verification of qualitative methods:
1) implementation verification (1 sample required)
2) food item verification (≥ 5 samples required)

Implementation verification (Minced meat)



S. Dublin Culture overnight (BHI 37°C, OD_{600nm}1), plating (1ml of 10^{-7},10^{-8}) and counting
Dilutions: A, B, C and D (4 replicates for each)

Estimated LOD_{50}

based on the number of positive results per level of contamination (protocol 1)

Table 6 — Determination of $eLOD_{50}$ based on the number of positive results per level of contamination using protocol 1

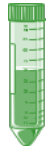
High inoculation level targeted 9 × LOD_{50}/ test portion	Intermediate inoculation level targeted 3 × LOD_{50}/ test portion	Low inoculation level targeted 1 × LOD_{50}/ test portion	Blank level	$eLOD_{50}$ cfu/test portion
1/1	4/4	4/4	0/1	< 1,0 × LIL[a]
1/1	4/4	3/4	0/1	= 0,5 × LIL
1/1	4/4	2/4	0/1	= 0,7 × LIL
1/1	4/4	1/4	0/1	= 1,0 × LIL

$eLOD_{50}$ 1x LIL=1 x 0,2=0,2cfu/25g acceptability limit $eLOD_{50}$ = 4 x 0,9=3,6 cfu/25g

Acceptability limits for implementation verification

the $eLOD_{50}$ is compared to the LOD_{50} value corresponding to the tested item

Food item verification (5 categories of food)



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Dilutions: A, B, C and D (4replicates for each)

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1/1	4/4	3/4	0/1	= 0,5 × LIL
1/1	4/4	2/4	0/1	= 0,7 × LIL
1/1	4/4	1/4	0/1	= 1,0 × LIL

Acceptability limits for food item verification:

the $eLOD_{50}$ shall not be > 4 x LOD_{50} observed in the validation study

Food item verification

Selection of food categories (+challenging!)

Classification of food categories for verification studies (Annex A - Table A.1)	Number of samples (internal data)
Multi-component foods or meat components	521
Raw meat and ready-to-cook meat products (except poultry)	391
Heat-processed milk and dairy products	255
Raw and ready-to-cook fish and seafoods (unprocessed)	245
Fresh procude and fruits	216
Raw poultry and ready-to-cook poultry products	178
Ready-to-eat, ready-to-reheat meat products	165
Raw milk and dairy products	64
Infant formla and infant cereals	61
Eggs and egg products (derivates)	49
Chocolate, bakery products and confectionary	41
Processed fruits and vegetables	35
Dried cereals, fruits, nuts, seeds and vegetables	21

Most frequently analyzed matrices in the service, 2018-2020

Description Food items (test portion 25g)

Food category	Type	Serotype	Challenge
Ready to eat food	Ready to eat salad	*S*. Dublin	Background flora, fat, acetic acid
Meat Products	Pork fresh meat	*S*. Typhimurium	Background flora
Meat Products	Poultry fresh meat	*S*. Kottbus	High background flora, higher pH than pork meat
Dairy products	Raw milk cheese	*S*. Dublin	High fat content (30%), low acidity, probiotics
Fishery	Mussels	*S*. Typhimurium	High background flora, high pH

Excel-calculation tool ISO 16140-3 ‘Method verification’

5.4.2 Inoculation of the test portions

Determination of the inoculum level (based on appropriate enumeration of the high-level inoculum, or by MPN according to Annex C)

	Implementation verification	(Food) item verification				
	Minced meet	Mixed salad	Raw pork chop	Raw poultry meat	Roquefort cheese	Raw mussels
Determined low inoculum level LIL (cfu/test portion):	0,2	0,6	0,3	0,4	0,3	0,2

5.5.1 Determination of $eLOD_{50}$ using protocol 1

Results per Inoculum level *[number of positive (food) item test portions per inoculum level: enter 0, 1, 2, 3 or 4 in each cell]*

Inoculum level of the test portions	(Food) item 1	(Food) item 2	(Food) item 3	(Food) item 4	(Food) item 5	(Food) item 6
High inoculum	1	1	1	1	1	1
Intermediate inoculum	4	2	3	2	3	3
Low inoculum	1	3	3	1	3	2
Blank (uninoculated)	0	0	0	0	0	0
$eLOD_{50}$ (cfu/test portion)	= 1,0 x LIL	= 1,5 x LIL	= 1,0 x LIL	= 2,6 x LIL	= 1,0 x LIL	= 1,3 x LIL
Determined $eLOD_{50}$ (cfu/test portion)	**0,2**	**0,9**	**0,3**	**1,0**	**0,3**	**0,3**

5.6 Acceptability limits (protocol 1)

The $eLOD_{50}$ shall not be > 4 x LOD_{50} observed in the validation study

Published validation data of the method. If no validation data is available, assume an LOD_{50} of 1 cfu/test portion

Observed LOD_{50} (cfu/test portion)	0,9	1,2	0,6	1,0	1,6	0,9
Acceptable $eLOD_{50}$ (cfu/test portion) = 4 * LOD_{50}	**3,6**	**4,8**	**2,4**	**4,0**	**6,4**	**3,6**
Acceptability limit evalution						
Determined $eLOD_{50}$ ≤ Acceptable $eLOD_{50}$	**Accepted**	**Accepted**	**Accepted**	**Accepted**	**Accepted**	**Accepted**

https://committee.iso.org/sites/tc34sc9/home/essential-information/content-left-area/validation-of-methods/method-validation-and-method-ver.html

Organises inter-laboratory comparative testing or proficiency tests between official laboratories

- Every 2 years (started in 2018)
- Matrix: chicken faeces
- Strain: *Salmonella* Agona
- About 10 participants, FSAFC approved labs
- About 10 samples
- Inoculation procedure: direct inoculation in 25g matrix → start analyse from de Stomacher bag
- 2 levels of contamination: low and high
- Transportation at fridge temperature
- Start analyses: the day after inoculation → fixed day
- Submission of results via web application
- Report

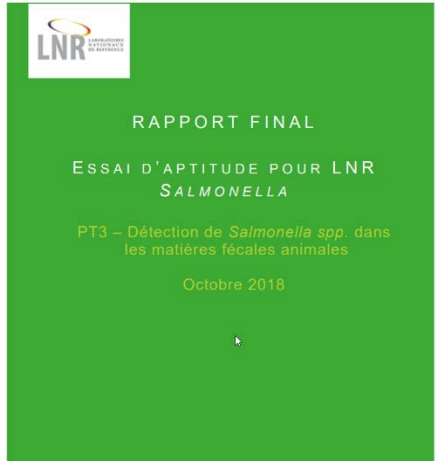

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Tableau 2 : Résultats des laboratoires

- 11 laboratories participated
- 6 samples
- 100% obtained results as expected

Nr. labo	Nr. d'échantillon					
	S1	S2	S3	S4	S5	S6
4	ND	D	D	D	D	ND
5	ND	D	D	D	D	ND
11	ND	D	D	D	D	ND
13	ND	D	D	D	D	ND
17	ND	D	D	D	D	ND
21	ND	D	D	D	D	ND
32	ND	D	D	D	D	ND
33	ND	D	D	D	D	ND
35	ND	D	D	D	D	ND
36	ND	D	D	D	D	ND
37	ND	D	D	D	D	ND

ND : non détecté/25g ; D : détecté/25g

diagnosis of outbreaks of foodborne-Salmonella cases

Food-borne outbreak

Health Inspection
(Aviq, Z&G, COCOM)

FASFC
(competent authority

Inquiry

Human samples

Inquiry

Food samples/
environment

NRL FBO (lead)
NRC Salmonella
NRL Salmonella

Analysis

Reporting of results

Laboratory investigation: Results food and environment

65 samples were taken: food leftovers, surfaces (kitchen, machines, tools, …)

matrix	qPCR screening *Salmonella*	ISO detection	serotype	MLVA type
Leftovers fish stick+ mashed potatoes + tartar sauce (25g)	Detected	Detected	Enteritidis	3-12-5-5-1
Fish stick (25g)				
Tartare sauce (25g)				
Mashed potatoes (25g)				
Chicken chops (garbage)				

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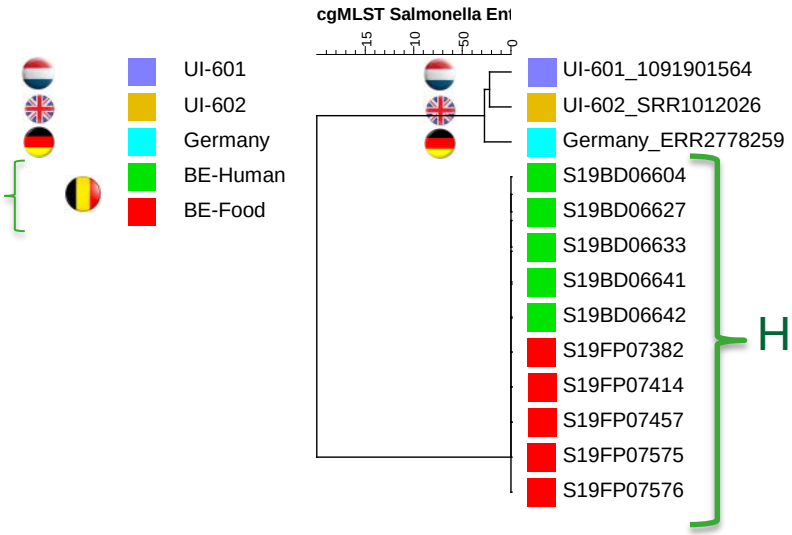
WGS analysis

Link to some EPIS or RASFF?

- UI-601: Spanish eggs (RASFF 2019-3069): Not related
- UI-602: UK eggs (RASFF 2019-1412): Not related
- German outbreak: eggs: Not related (MLVA 2-10-7-3-2)



(>170 cgMLST differences)

Human isolates BE identical to food isolates

Sciensano	FAVV	Serotypering	AMR genes	Plasmid replicon	MLST
S19FP07382	1882-19-0071	*Salmonella* Enteritidis	aac(6')-Iaa	IncFIB, IncFII	11
S19FP07414	2539-19-0094	*Salmonella* Enteritidis	aac(6')-Iaa	IncFIB, IncFII	11
S19FP07457	2205-19-0003	*Salmonella* Enteritidis	aac(6')-Iaa	IncFIB, IncFII	11
S19FP07575	2205-19-0008	*Salmonella* Enteritidis	aac(6')-Iaa	IncFIB, IncFII	11
S19FP07576	2205-19-0009	*Salmonella* Enteritidis	aac(6')-Iaa	IncFIB, IncFII	11

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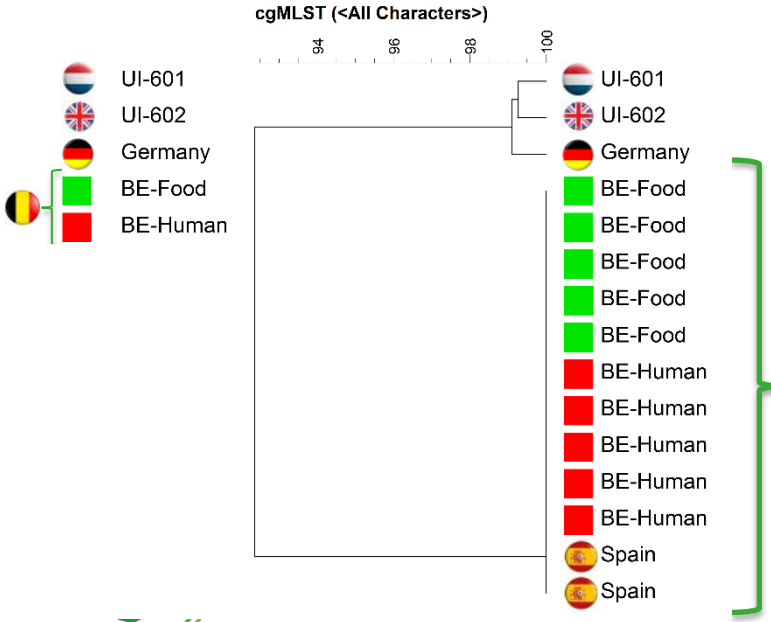
Trace back analysis: outcome

- UI-601: Spanish eggs (RASFF 2019-3069):
  Not related (>170 cgMLST differences)
- UI-602: UK eggs (RASFF 2019-1412): Not related
- German outbreak: eggs: Not related
- Spanish eggs: 2 isolates May & November 2019 (UI-608)



Egg farm Spain
Warehouse and packaging centre
↓
Exported to BE August 2019

Human and food isolates BE identical to isolates from Spain

ST11 CT3903

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Ensure the dissemination to the competent authorities and official laboratories of information that the European Union reference laboratory supplies

- Participation in EURL-Salmonella Workshop
- Dissemination of the content of the workshop to the national authority

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FAVV
DG Laboratoria
Kruidtuinlaan 55
1000 Brussel

Bruxelles, le 3 juin 2021

Concerne : Rapport d'activités du 26ème workshop virtuel de l'EURL *Salmonella*, le 28 Mai 2021

Rapporteurs : Cristina Garcia-Graells (Sciensano)
Rapport d'activités du workshop virtuel organisé par l'EURL-*Salmonella*

training courses for the staff of official laboratories designated

- Training Poulpharm: ISO 6579-1:2017
- Participation in the national working group Salmonella in the poultry sector
- Participation in ISO revision part 4: NRC-Salmonella

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DEELNEMERS CERTIFICAAT

Organisator

SCIENSANO – NRL SALMONELLA

Workshop

Detectie van Salmonella species in stalen afkomstig van de primaire productie

Datum

02 DECEMBER 2019

Naam deelnemer en organisatie

Poulpharm

Naam en handtekening van de organisator

Namens NRL,

Koenraad Van Hoorde

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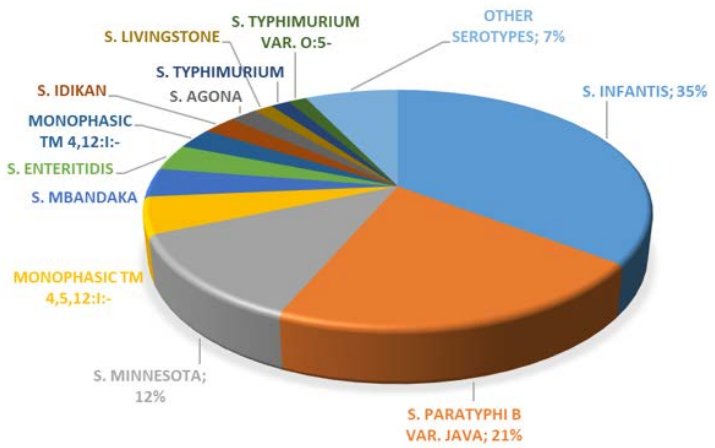
Poultry: overview of isolated serotypes in 2019



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NRL Northern Ireland

In Northern Ireland (UK), EU law continues to apply post EU exit in respect of the majority of food and feed hygiene law, as listed in the Northern Ireland Protocol (NIP).

This includes the Official Control Regulation (EU) 2017/625 for Official Feed and Food Controls.

The Northern Ireland Protocol has specific requirements for the designation of NRLs in respect to Northern Ireland (paragraph 43, annex 2):

43. **Official controls, veterinary checks**

References to national reference laboratories in the acts listed in this section shall not be read as including the reference laboratory in the United Kingdom. This shall not prevent a national reference laboratory located in a Member State from fulfilling the functions of a national reference laboratory in respect of Northern Ireland. Information and material exchanged for that purpose between the competent authorities of Northern Ireland and a national reference laboratory in a Member State shall not be subject to further disclosure by the national reference laboratory without the prior consent of those competent authorities.

— Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation) ([249])

Official Laboratories NI

2 OL located within NI:

- Agri-Food and Biosciences Institute (AFBI)
- The Northern Ireland Public Health Lab (NIPHL)

FSA has also designated OL within MS, which are run by Eurofins and as such they rae also under other MS NRLs.

The UK has designated NRLs which also provide support to the OLs of NI

For Microbiology: Public Health England

Requirements for NI NRLs

The responsibilities and tasks for NRLs are laid out under Article 101 of the EU Official Control Regulation 2017/625.

In accordance with the Northern Ireland Protocol (NIP), the FSA has designated laboratories based in EU Member States to provide required NRL functions under Regulation 2017/625 for Northern Ireland. However, Northern Ireland may still refer to UK NRLs for other functions such as assistance with technical questions and support with proficiency testing beyond that provided by NI specific NRLs.

Therefore, not all of the responsibilities and tasks are needed to the same degree, given the UK's existing NRL system. The most relevant requirements are:

- Collaborate with the EURLs, and participate in training courses and in inter-laboratory comparative tests organised by these laboratories;
- Ensure the dissimination to the competent authorities and official laboratories of information that the European Union reference laboratory supplies;
- Where appropriate, organise inter-laboratory comparative testing or proficiency tests between official laboratories, ensure an appropriate follow-up of such tests and inform the competent authorities of the results of such tests and follow-up;
- Where necessary, conduct training courses for the staff of official laboratories designated under Article 37(1);
- Coordinate the activities of official laboratories designated in accordance with Article 37(1) with a view of harmonising and improving the methods of laboratory analysis, test or diagnosis and their use

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