



EURL Lm

European Union Reference Laboratory for
Listeria monocytogenes
<http://eurl-listeria.anses.fr>

IDENTIFICATION AND DETECTION BY REAL-TIME PCR OF 30 MAJOR CIRCULATING *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES IN EUROPE

Benjamin FELIX

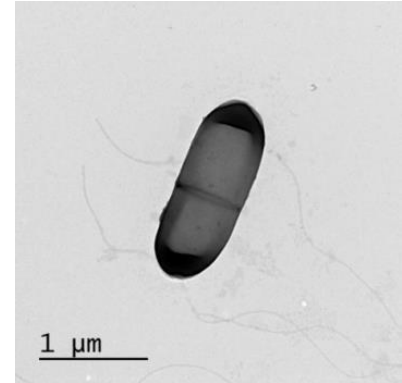
benjamin.felix@anses.fr

European Union Reference Laboratory for *Lm*

1 — Context



- Listeriosis* :
 - Vehicle: contaminated food product
 - Rare disease (0,49 cases per 100,000 people)
 - First foodborne disease for hospitalisation rate 96.5%, and death with 196 reported in the EU
- Ubiquitous : water, soil, plant, animal/human, gut asymptomatic carriage is common**
- Contaminant of all food processing facilities, psychrotroph and persistence abilities



Anses Lab for food Safety,
Boulogne sur Mer location



Thisted Lambertz 2014

* : EFSA-ECDC zoonosis report 2021 doi.org/10.2903/j.efsa.2022.7666

** : Hafner et al. 2021 doi.org/10.1038/s41467-021-27069-y

2 — *L. monocytogenes* typing



Multi Locus Sequence Typing (MLST) principle

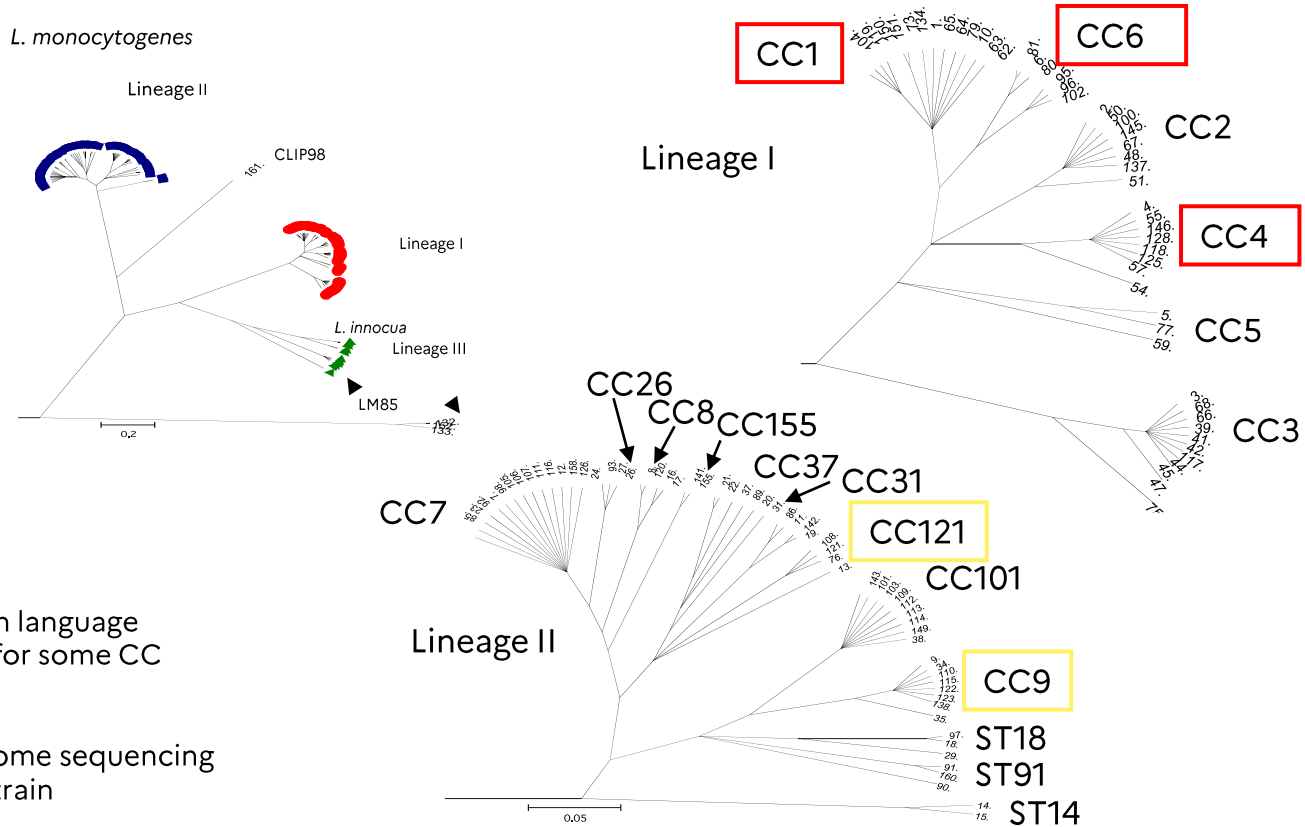


- Sequencing of 7 housekeeping genes
- *abcZ* (transporteur ABC)
- *bglA* (β-glucosidase)
- *cat* (catalase)
- *dapE* (succinyl diaminopimelate desuccinylase)
- *dat* (transférerase)
- *ldh* (lactate déshydrogénase)
- *hikA* (histidine kinase)

CC : 6 alleles in common
ST : 7 alleles in common

To the 19/09/2023: <https://bigsd.b.pasteur.fr/>

- Number of ST: 3149
- Number of CC : 261
- ST not assigned to a CC: 1050



Universal & common language
Relate to virulence for some CC

MLST by whole genome sequencing
Approx. 150 € per strain
3-5 days

From Ragon et al. 2008, PLoS Pathogens

2 — Method design



EURL-NRL for *Lm* sequencing studies



MICROBIAL GENOMICS

BIORESOURCE



Painc et al. Microbial Genomics 2019:5

ORIGINAL RESEARCH
published: 02 November 2021
doi: 10.3389/fmicb.2021.729050

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ELSEVIER



veterinary sciences

Papić et al. BMC Microbiology (2019) 19:
<https://doi.org/10.1186/s12866-019-1676-3>

Article
Char
genes

RESEARCH ARTICLE

Genetic diversity of *Listeria monocytogenes* strains in ruminants and rhombencephala with the natural

Bojan Papić^{1*}, Mateja Pate¹, Benjamin Félix et al.[#]

Genetic diversity of *Listeria monocytogenes* products in

Elżbieta Maćkaj
Katarzyna Kuś

^a Department of Food Safety and Food Quality
^b European Union Reference Laboratory for Listeria

Žaneta Šušteršič
Silva Grašič

scientific data

OPEN

DATA DESCRIPTOR

A European-wide dataset to uncover adaptive traits of *Listeria monocytogenes* to diverse ecological niches

Benjamin Félix et al.[#]

Listeria monocytogenes (*Lm*) is a ubiquitous bacterium that causes listeriosis, a serious foodborne illness. In the nature-to-human transmission route, *Lm* can prosper in various ecological niches. Soil and decaying organic matter are its primary reservoirs. Certain clonal complexes (CCs) are over-represented in food production and represent a challenge to food safety. To gain new understanding of *Lm* adaptation mechanisms in food, the genetic background of strains found in animals and environment should be investigated in comparison to that of food strains. Twenty-one partners, including food, environment, veterinary and public health laboratories, constructed a dataset of 1484 genomes originating from *Lm* strains collected in 19 European countries. This dataset encompasses a large number of CCs occurring worldwide, covers many diverse habitats and is balanced between ecological compartments and geographic regions. The dataset presented here will contribute to improve our understanding of *Lm* ecology and should aid in the surveillance of *Lm*. This dataset provides a basis for the discovery of the genetic traits underlying *Lm* adaptation to different ecological niches.

Background & Summary

LiSEQ of *Listeria monocytogenes* in clinical samples

Anaïs Painc
Corinne Ancelet
Marc Lecuit

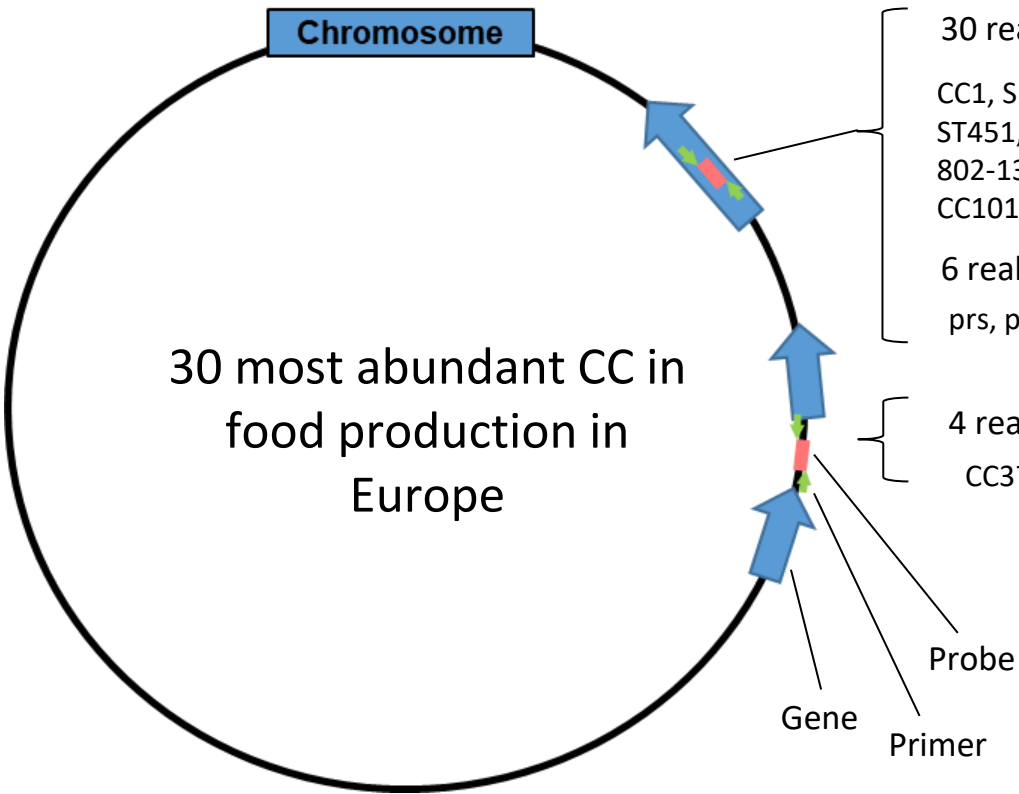
harness the biodiversity of the microbiome

Unpublished contribution of the National Reference Laboratory (NRL) CH, CZ, DE, IT, NL, SE

Real-time PCR assay based on *Lm* circulating diversity in food



40 primer-probe real-time PCR assays



30 real-time PCR assays targeting the gene-coding sequences

- CC1, SD-CC1, CC2, CC3, CC4, CC5, CC6, CC7, CC8, CC9, CC11-ST451, CC14-ST14-206-399, CC14-ST91-360, CC18, CC19-ST398-802-1308, CC20, CC21, CC26, CC29, CC31, CC54, CC59, CC77, CC101, CC121, SD-CC121, CC155, CC199, CC204, CC224

6 real-time PCR for molecular serotyping (Vitullo et al. 2013)

- prs, plca, Lmo0737, Lmo1118, ORF2819, ORF2110

4 real-time PCR assays targeting the intergenic regions

- CC37, SD-CC37, CC87, CC193

Available in:
(i) High throughput 40 simplex
(ii) Multiplex 11 triplex, 1 duplex



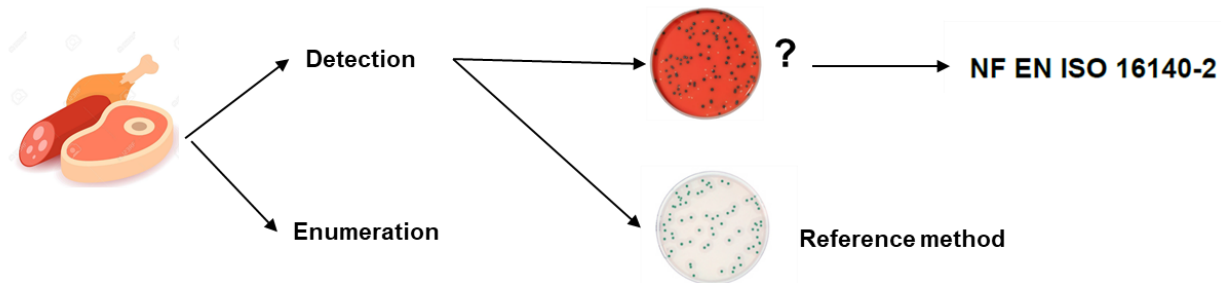
In silico analysis on 3342 genomes, from 18 EU & 19 non-EU countries

SD = sub-division, PCR assay splitting a CC into two groups

3 — Method validation



Validation of alternative method against a reference method in food microbiology



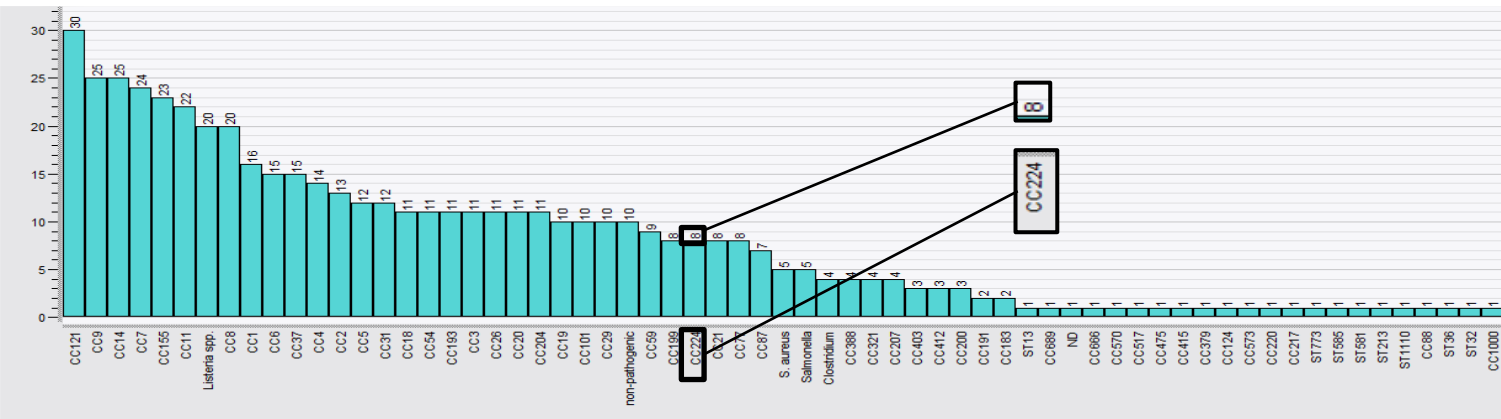
Part 4 : sensibility and limit of detection

Partie 6 : dedicated to alternative typing method



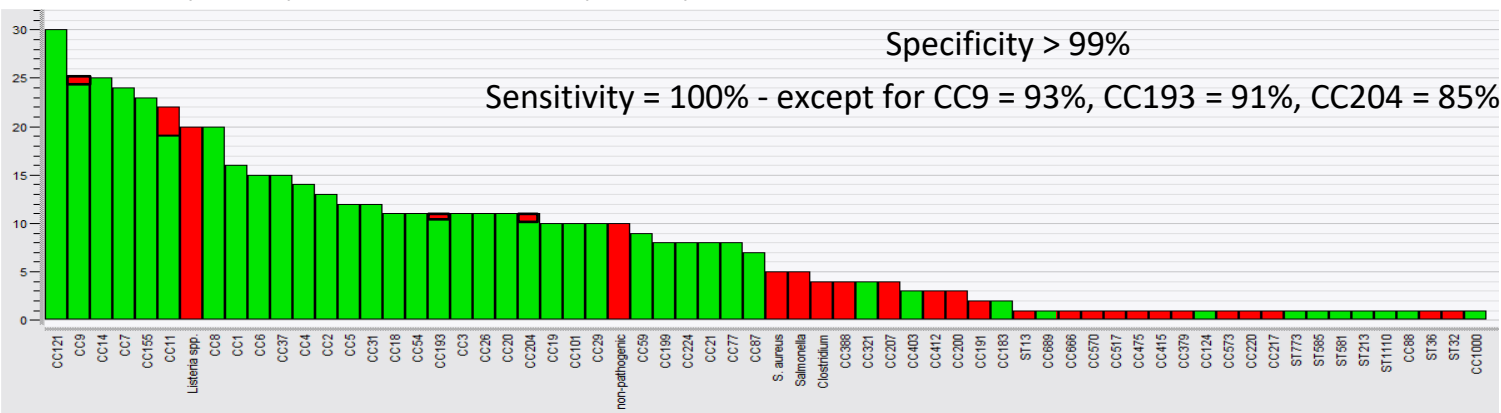
Validation panel: sequenced strains (n=597)

Whole validation panel diversity (n=597)



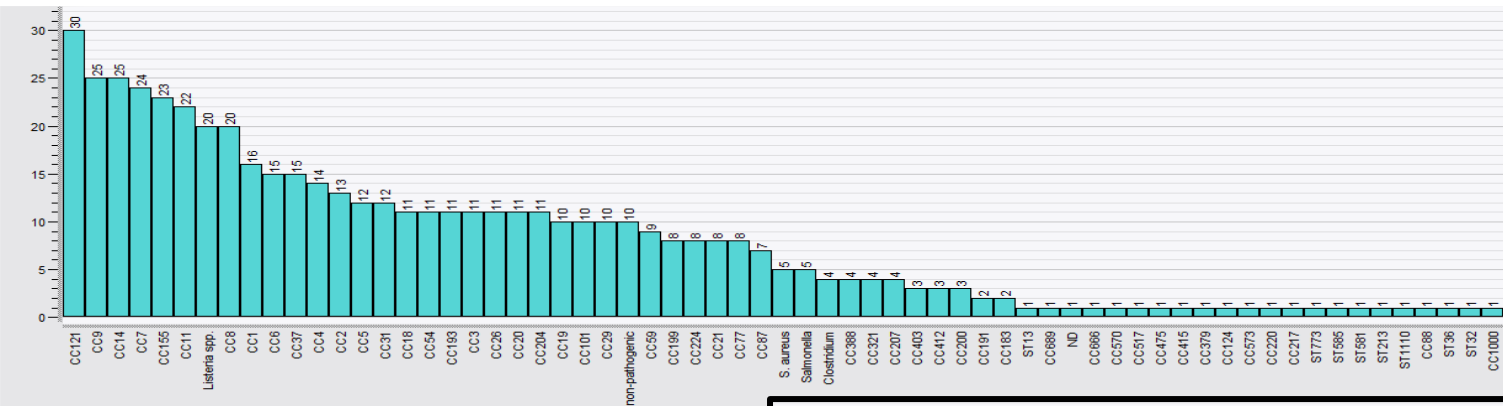
Isolates from 21 EU countries

Identified (n=480) and Not identified (n=117)

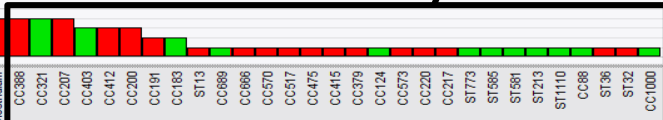
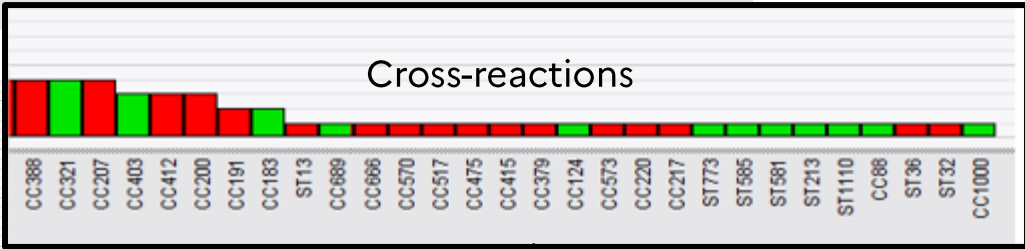
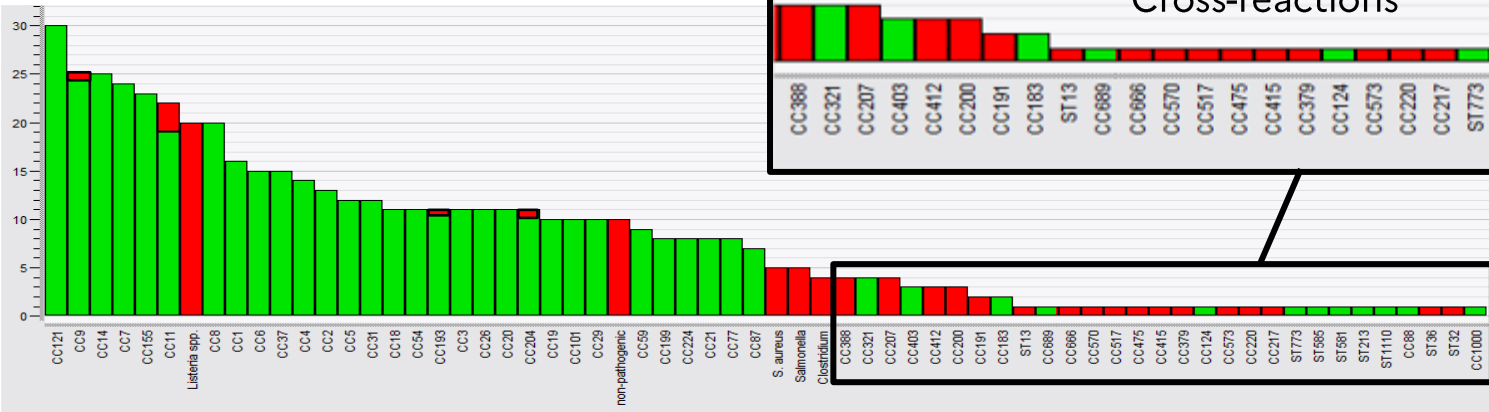


Validation panel: sequenced strains (n=597)

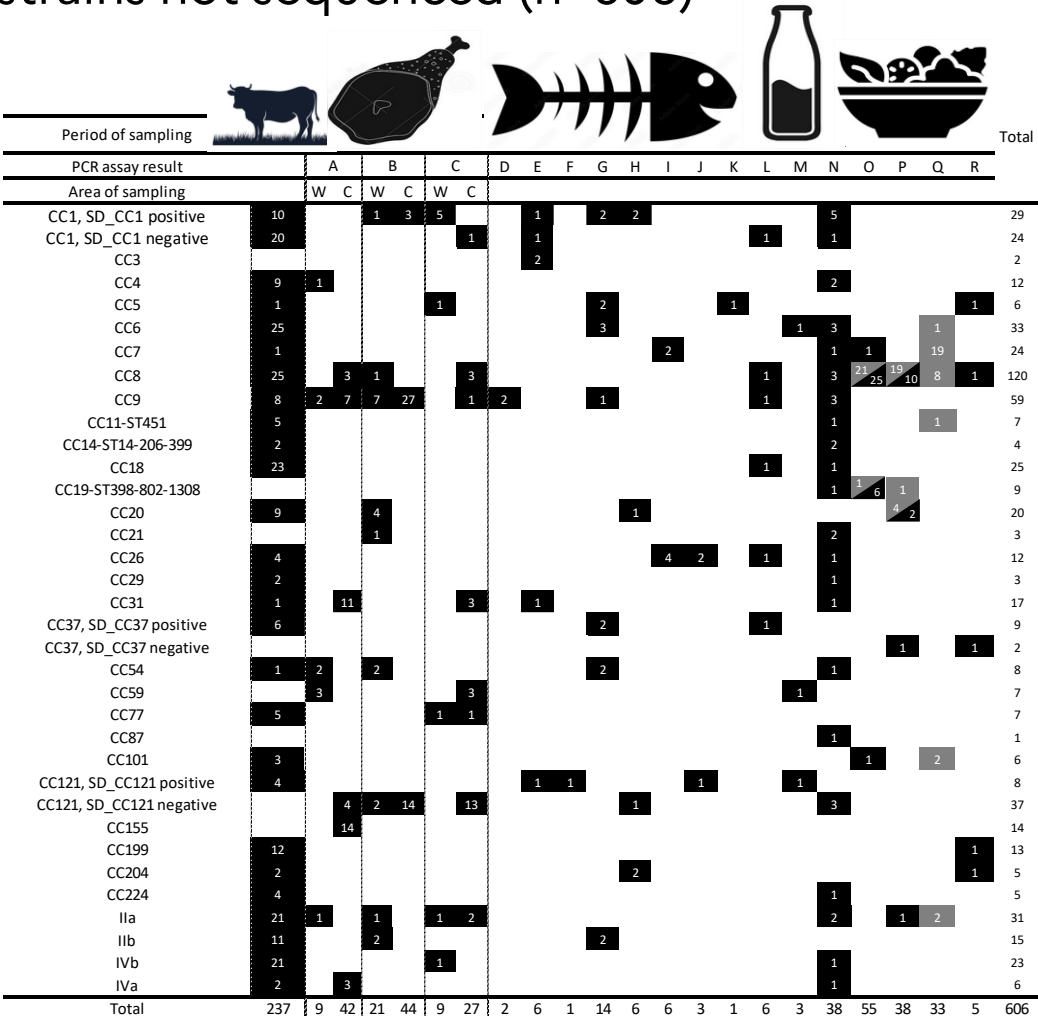
Whole validation panel diversity (n=597)



Identified (n=480) and Not identified (n=117)



Performance: strains not sequenced (n=606)



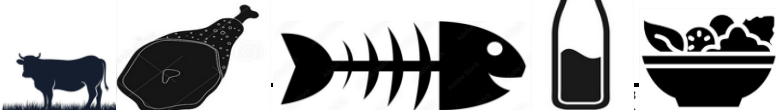
Major food sectors at risk for *Lm*

Period 2006-2022

Strains from 18 processing plant environments

Strains from ruminant or raw milk production environments

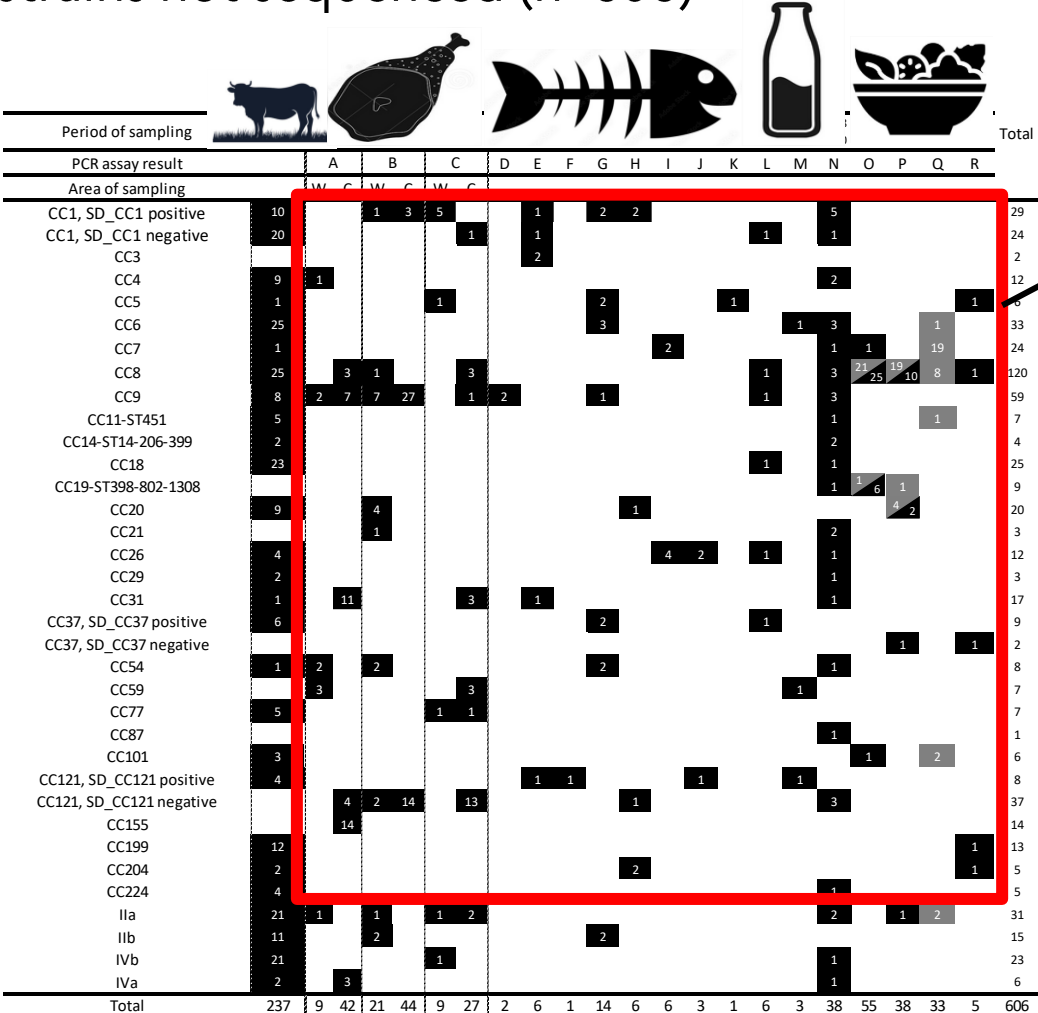
Performance: strains not sequenced (n=606)



Ruminant, farm
78% coverage

Area of sampling	Period of sampling																				Total		
	A		B		C		D	E	F	G	H	I	J	K	L	M	N	O	P	Q		R	
	W	C	W	C	W	C																	
CC1, SD_CC1 positive	10		1	3	5			1		2	2						5					29	
CC1, SD_CC1 negative	20					1		1							1		1					24	
CC3								2									2					2	
CC4	9	1															2					12	
CC5	1				1					2				1							1	6	
CC6	25									3						1	3				1	33	
CC7	1											2					1	1	1	19		24	
CC8	25	3	1			3								1			3	24	25	10	8	1	120
CC9	8	2	7	7	27	1	2			1				1			3						59
CC11-ST451	5																1				1	7	
CC14-ST14-206-399	2																2					4	
CC18	23															1						25	
CC19-ST398-802-1308																	1	6	1			9	
CC20	9			4							1										4	20	
CC21				1													2					3	
CC26	4												4	2			1					12	
CC29	2																					3	
CC31	1		11			3		1														17	
CC37, SD_CC37 positive	6								1		2					1						9	
CC37, SD_CC37 negative																				1		1	2
CC54	1	2		2							2											8	
CC59		3					3									1						7	
CC77	5					1	1															7	
CC87																					1	1	
CC101	3																			1		2	6
CC121, SD_CC121 positive	4							1	1								1					8	
CC121, SD_CC121 negative			4	2	14		13				1										3		37
CC155			14																				14
CC199	12																					1	13
CC204	2										2											1	5
CC224	4																				1		5
IIa	21	1		1		1	2														2		31
IIb	11			2						2													15
IVb	21					1																	23
IVa	2			3																	1		6
Total	237	9	42	21	44	9	27	2	6	1	14	6	6	3	1	6	3	38	55	38	33	5	606

Performance: strains not sequenced (n=606)



Processing plant
94% coverage

Performance: strains not sequenced (n=606)

Period of sampling	Area of sampling																			Total			
PCR assay result	A		B		C		D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		
Area of sampling	W	C	W	C	W	C																	
CC1, SD_CC1 positive	10			1	3	5			1		2	2					5					29	
CC1, SD_CC1 negative	20						1		1							1	1					24	
CC3								2									2					2	
CC4	9	1															2					12	
CC5	1				1					2				1						1		6	
CC6	25									3						1	3					33	
CC7	1											2					1	1	1	19		24	
CC8	25		3	1			3								1		3	24	25	10	8	1	120
CC9	8	2	7	7	27		1	2		1					1		3					59	
CC11-ST451	5																1				1	7	
CC14-ST14-206-399	2																2					4	
CC18	23															1						25	
CC19-ST398-802-1308																	1	6	1			9	
CC20	9			4							1									4		20	
CC21				1																2		3	
CC26	4											4	2			1						12	
CC29	2																			1		3	
CC31	1		11				3		1													17	
CC37, SD_CC37 positive	6								1		2					1						9	
CC37, SD_CC37 negative																			1		1	2	
CC54	1	2		2							2											8	
CC59		3					3									1						7	
CC77	5					1	1															7	
CC87																				1		1	
CC101	3																			1	2	6	
CC121, SD_CC121 positive	4							1	1				1				1					8	
CC121, SD_CC121 negative			4	2	14		13				1									3		37	
CC155			14																			14	
CC199	12																					13	
CC204	2										2											5	
CC224	4																					5	
IIa	21	1		1		1	2											2		1	2	31	
IIb	11			2						2												15	
IVb	21					1																23	
IVa	2		3																	1		6	
Total	237	9	42	21	44	9	27	2	6	1	14	6	6	3	1	6	3	38	55	38	33	5	606

Not covered were sequenced
 CC11-ST11, ST191,
 CC207, CC217,
 CC379, CC415,
 CC517 and CC991

4 — Why not to apply it for detection ?

Direct CC detection

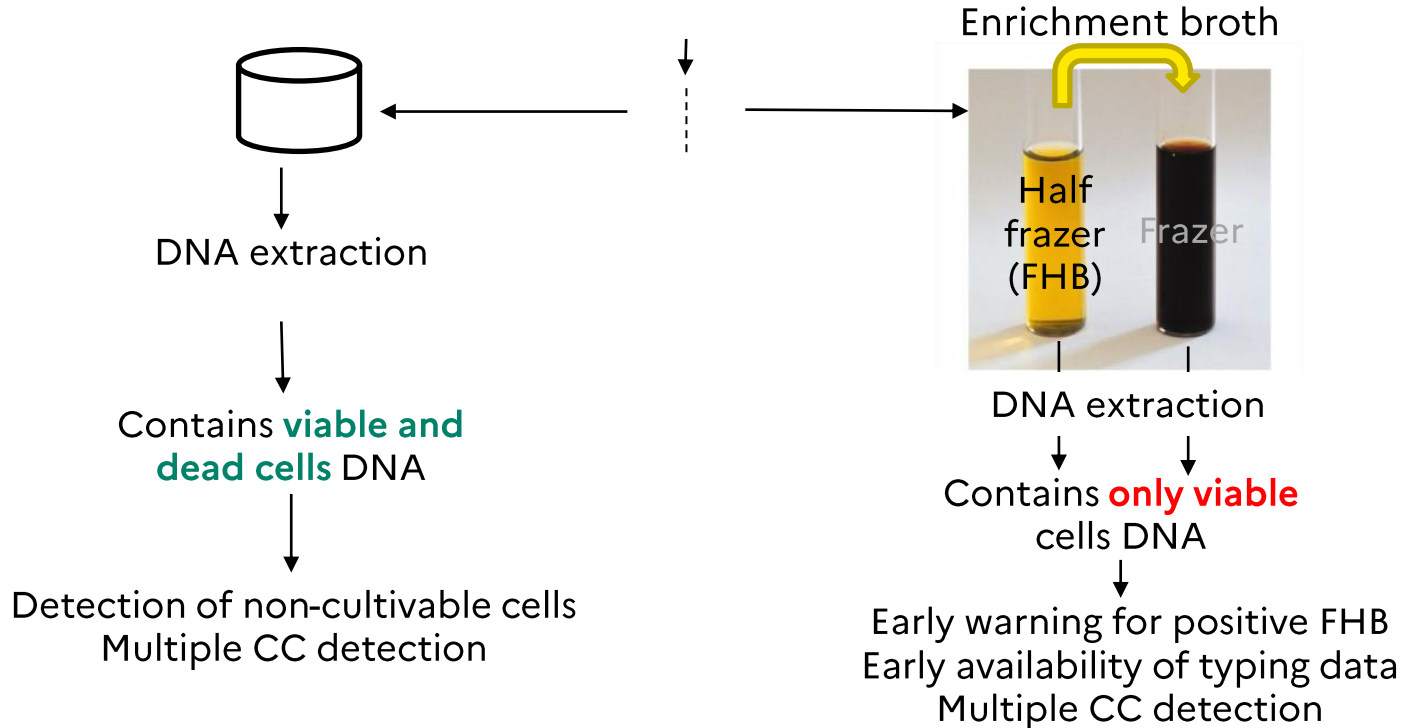
Soil sample



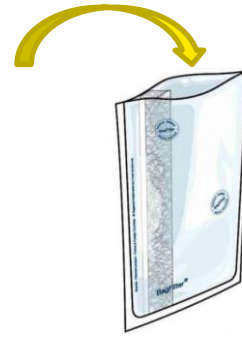
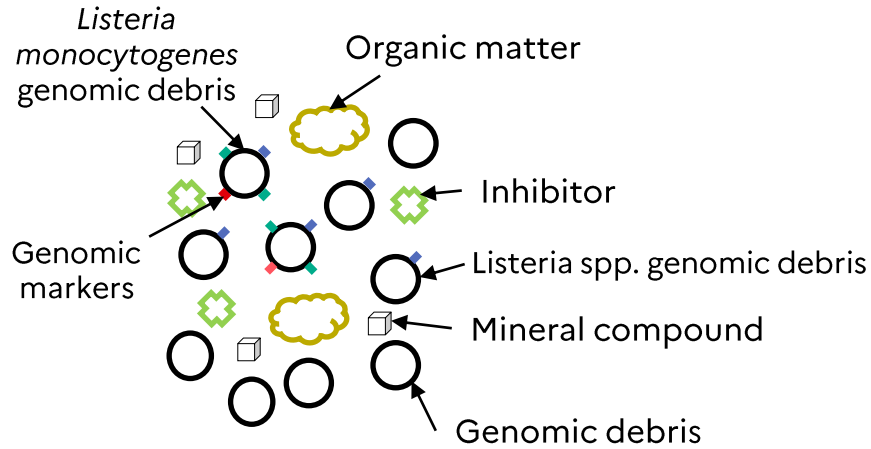
... Various contaminated samples ...



Animal sample



Bead beating extraction principle



Half Fraser broth (HFB)



Animal sample



Photo origin google pictures

Soil

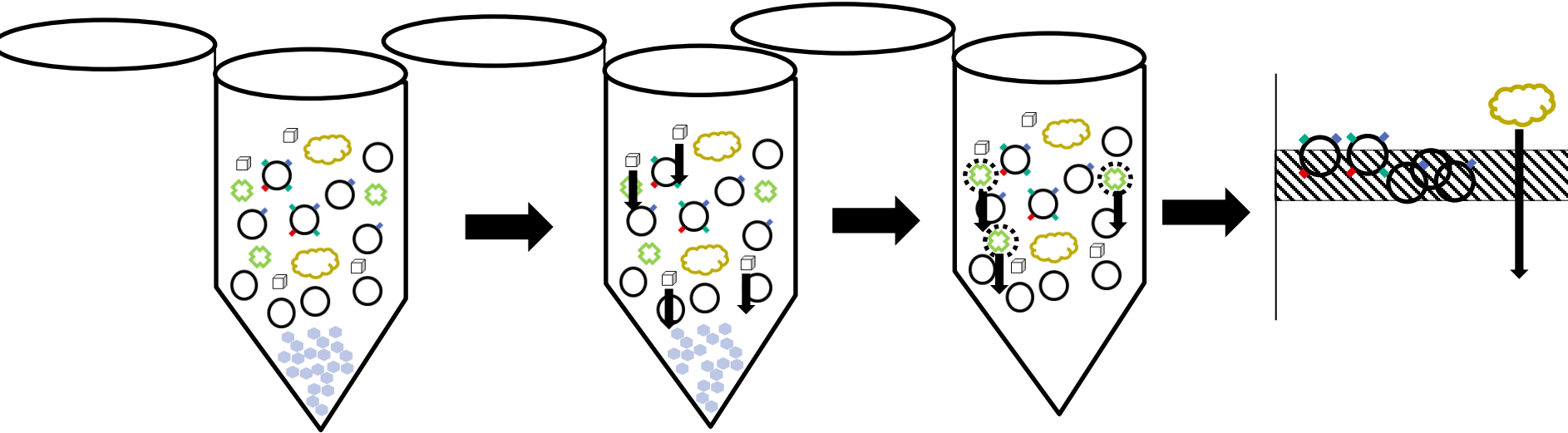
Kit used:

- QIAGEN DNeasy power soil pro
- QIAGEN DNeasy power food
- iHelix (<https://www.ihelix.eu/>)

- ◆◆◆ Clonal complex
- ◆ Molecular serotype
- ◆ Genus marker

Bead beating extraction principle

- Bead beating step



- Break soil compound
- Break micro-organism cell walls
- Homogenise

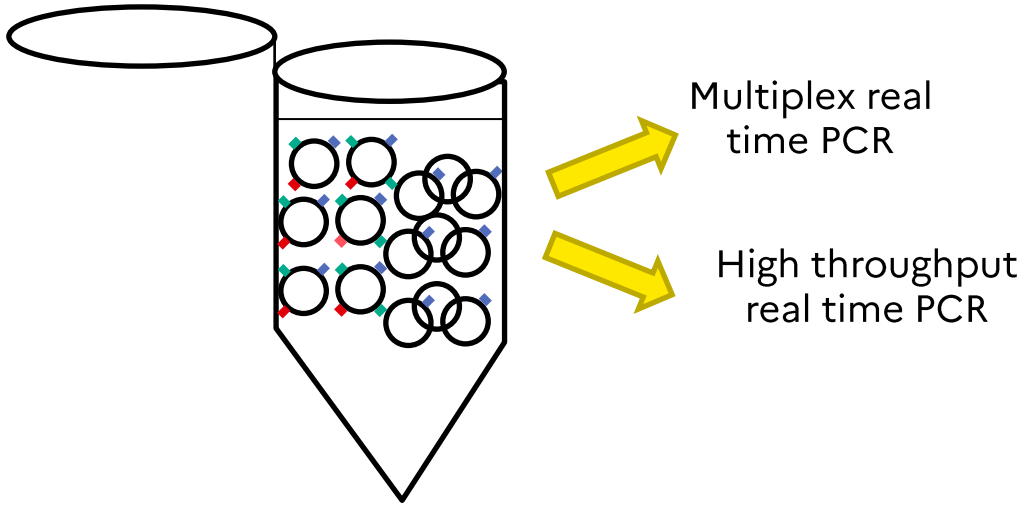


- Sedimentation by centrifugation

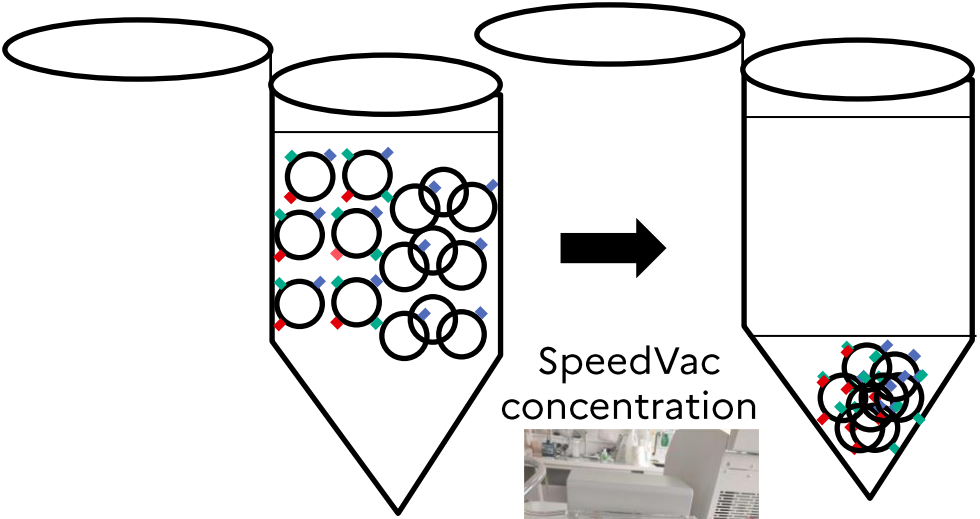
- Inhibitor removal technology, catch inhibitor and not DNA

- Purification and washing by elution on silica membrane

CC detection by real time PCR



DNA concentration and amplification for trace detection

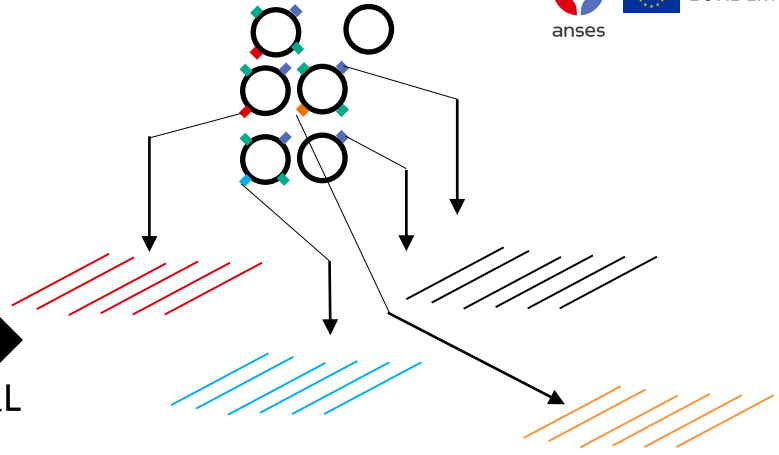


SpeedVac concentration



X10 concentration

5 μ L



X 14,000
Total volume
50 μ L

Pre-amplification by 40 simultaneous polymerase chain reaction (PCR)



Real Time PCR for CC detection

6 – Validation



Sensitivity – experimental plan

Reference method

GenoListeria detection

Half Fraser broth (HFB)

Isolation on ALOA selective medium

Pure strain

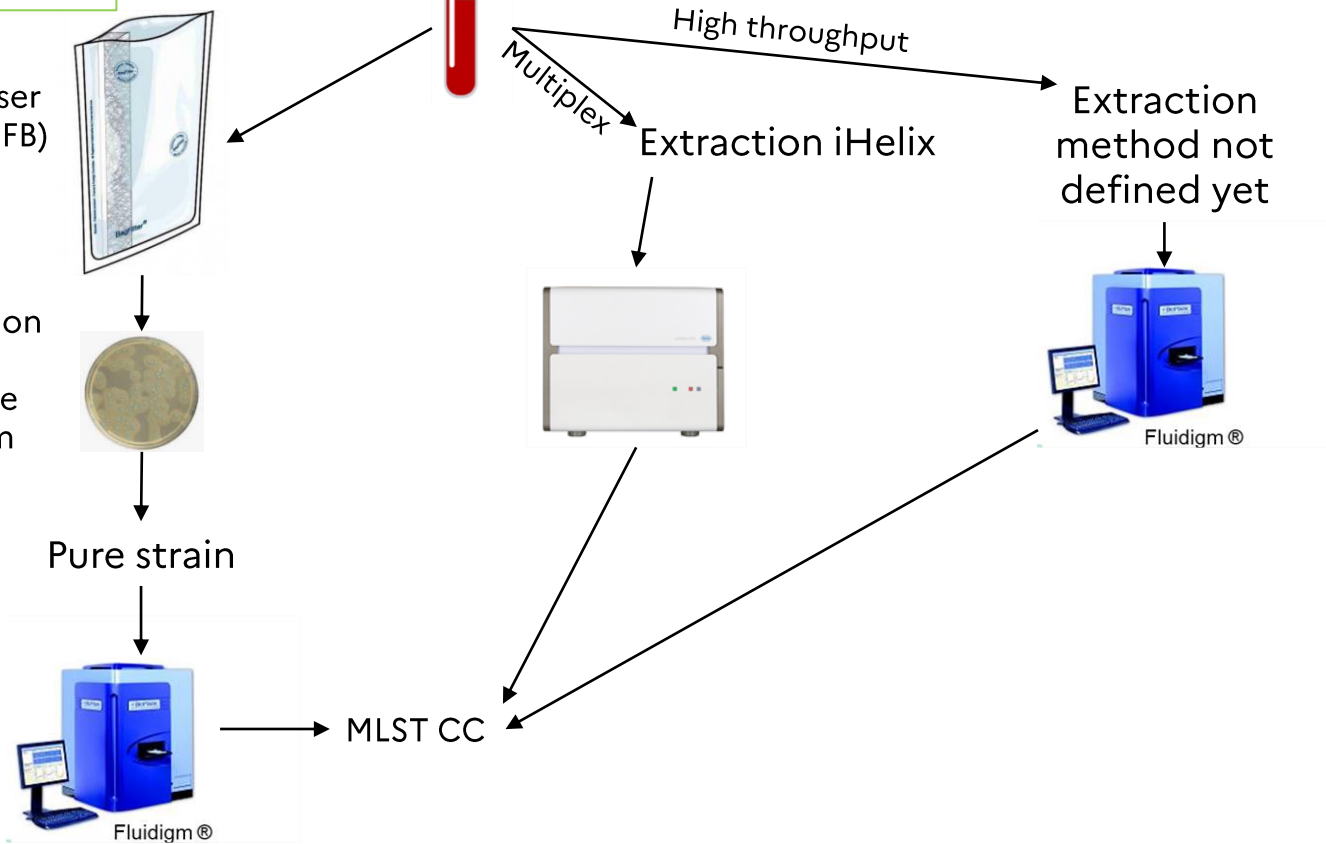
Naturally contaminated sample

Multiplex

High throughput

Extraction iHelix

Extraction method not defined yet



Results





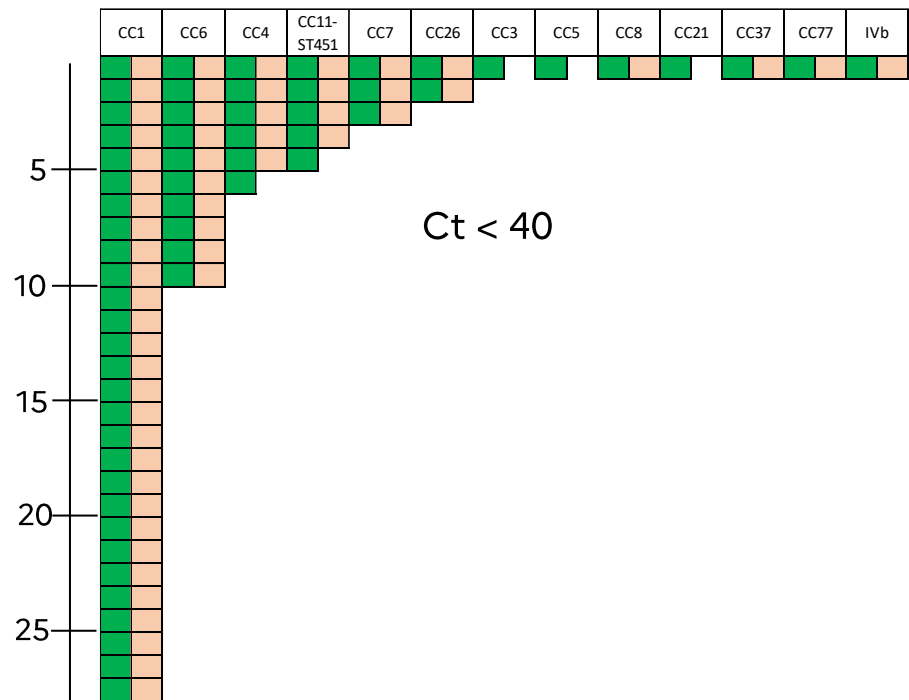
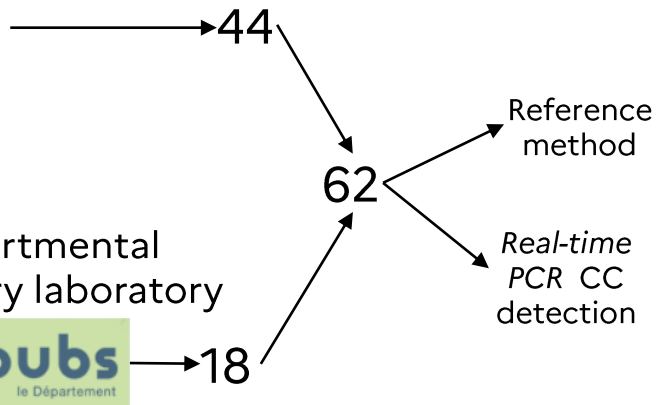
University
of Ljubljana *Veterinary*
Faculty



Sensitivity (preliminary results)



Placenta naturally
contaminated
samples



■ Reference method – enrichment Fraser 1/2 and Fraser + GenoListeria on pure strains
■ Direct real time PCR CC detection

Limit of detection (preliminary results)

Homogenised
placenta sample



Preliminary results let show a
 LOD_{95} between 100 and 1000
CFU/mL

CC detection:

On pure strain

Summary: ANSES was accredited ISO16140/17025 in 2021 and published the method*.

Aim: Discriminant, rapid, cost effective, informative, broadly accessible, frontline method, scalable and open

In soil sample

In progress – application on research contaminated pasturage, sludge, river sediment

In placenta sample

Summary: Sensitivity analysis in progress (nine CC PCR tested already). Good method sensitivity, presumptive LOD₉₅ between 100 and 1000 CFU/mL.

Aim: detect multiple contaminations, detect cross contamination between samples.

* Félix et al. 2022. Identification by high-throughput real-time PCR of 30 *Listeria monocytogenes* Clonal Complexes. *Spectrum in Microbiology*, doi: 10.1128/spectrum.03954-22

In placenta sample

Extend sensitivity panel to reach at least one strain per targeted PCR, finalize LOD tests. Publication on *Lm* genetic diversity related to ruminant abortion.

In soil sample

Collaborative project (not defined yet)

CC detection on food using selective medium (Fraser half broth)

One day typing method, multi contamination, study strain on the competition in selective medium

CC detection in samples from food processing plants

High frequency sampling, multi contamination, trace contamination in processing plant

Anses Lab for Food Safety,

Maisons-Alfort location:

1- SEL unit

- Corinne Danan
- Sophie Roussel
- Karine Capitaine
- Florence Va
- Delphine Fert
- Adrien Asséré
- Sabrina Cadel-Six

2- IdentityPath platform

- Patrick Fach
- Sabine Delannoy
- Mai-Lan Tran

Anses NGS platform

Bio-informatic team

- Arnaud Felten

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NRLs Lm Working group:

BE, CY, CH, DE, IT,

NLrvm, NLwfsr, PT, SE,

SI, SK

Veterinary faculty of

Ljubljana

- Bojan Papić
- Darja Kušar
- Maja Kavalič
- Jana Avberšek

Departmental veterinary
laboratory (N°25)

- Marc Hessemann
- Fanny Terraz-Laderriere

Anses Lab for Food Safety,
Boulogne-sur-Mer location

B3PA unit

- Graziella Bourdin
- Thomas Brauge
- Guylaine Leleu

ADRIA Quimper

- Guillaume Gillot
- Nassim Mouhali

Institute for pig and
pork industry

- Carole Feurer

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University
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Faculty

