

The activities of the Italian National Reference Laboratory (NRL) of *Listeria monocytogenes*

Marina Torresi

WOAH CC FH, IZS-Teramo

National Reference Laboratory for Listeria monocytogenes

ERFAN Laboratory training course 17-21 October 2022



National Reference Laboratory



At European level Member states designate national reference laboratories according to Article 100 of Regulation (EU) No 625/2017

Member States shall designate one or more national reference laboratories for each European Union reference laboratory designated. Member States that have more than one national reference laboratory for a European Union reference laboratory shall ensure that such laboratories work closely together, so as to ensure efficient coordination between them, with other national laboratories and with the European Union reference laboratory.

A Member State may designate a laboratory situated in another Member State or in a third country that is a Contracting Party to the Agreement on the European Economic Area.

A single laboratory may be designated as a national reference laboratory for more than one Member State.

The Ministry of Health appointed the IZSAM as National Reference Laboratory for *Listeria monocytogenes* in 2007.

A laboratory can be designated as NRL if it:

- a) has the expertise, equipment and infrastructure required to carry out analyses or tests or diagnoses on samples;
- b) has a sufficient number of suitably qualified, trained and experienced staff;
- c) ensures that the tasks are performed impartially and which is free from any conflict of interest;
- d) can deliver in a timely manner the results of the analysis, test or diagnosis carried out on the samples taken during official controls and other official activities;
- e) operates in accordance with the standard EN ISO/IEC 17025 and is accredited in accordance with that standard by a national accreditation body

The National reference laboratory shall:

- a) be impartial, free from any conflict of interests, and in particular not be in a situation which may, directly or indirectly, affect the impartiality of their professional conduct as regards the exercise of their tasks as national reference laboratories;
- b) have, or have contractual access to, suitably qualified staff with adequate training in analytical, testing and diagnostic techniques in their area of competence;
- c) possess, or have access to, the infrastructure, equipment and products needed to carry out the tasks assigned to them;
- d) ensure that their staff and any contractually engaged staff have good knowledge of international standards and practices and that the latest developments in research at national, Union and international level are taken into account in their work;
- e) be equipped with, or have access to, the necessary equipment to perform their tasks in emergency situations;
- f) where relevant, be equipped to comply with relevant biosecurity standards.

Responsibilities and tasks of national reference laboratories

National reference laboratories shall, in their area of competence:

- a) collaborate with the European Union reference laboratories, and participate in training courses and in inter-laboratory comparative tests organised by these laboratories;



National reference laboratories shall, in their area of competence:

- b) coordinate the activities of official laboratories with a view of harmonizing and improving the methods of laboratory analysis, test or diagnosis and their use;
- c) organize 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;

IZS Teramo
Vers. 1.0 Build 6
Prove Interlaboratorio IZSAM

Listeria monocytogenes

Per poter accedere al servizio, selezionare l'Organizzazione ed inserire la password

Organizzazione/Laboratorio:
I.Z.S. ABRUZZO E MOLISE - TERAMO

Password:

Accedi

Protocolli per l'esecuzione di prove valutative tra laboratori 2012

Reports finali
N° distrib. 06/Lm
N° distrib. 05/Lm
N° distrib. LM-2015
N° distrib. LM-2014
N° distrib. LM-2013
N° distrib. LM-2012

National reference laboratories shall, in their area of competence:

- d) ensure the dissemination to the competent authorities and official laboratories of information that the European Union reference laboratory supplies;

**GIORNATA DI STUDIO DEL
LABORATORIO NAZIONALE DI RIFERIMENTO PER
LISTERIA MONOCYTOGENES (LNR LISTERIA M.)
27 ottobre 2022**



RESPONSABILE SCIENTIFICO

Francesco Pomilio
Responsabile Reparto
Igiene e Tecnologie
degli Alimenti
IZSAM

DATA: 27/10/2022

MODALITÀ: Webinar

ORA: dalle 9.00 alle 12.00

LINGUE: Italiano/Inglese

DOVE: Il webinar sarà erogato attraverso la piattaforma Cisco Webex Events. Il link di accesso sarà fornito dopo l'iscrizione all'evento.

MODALITÀ ISCRIZIONE: <http://formazione.izs.it>

RISPONDE A ...

La finalità principale dell'evento è quella di informare la rete dei laboratori ufficiali, di altri istituti di diagnosi e ricerca e vari portatori di interesse sulle attività del LNR per *Listeria monocytogenes* e sulle novità provenienti dall'EURL Lm.

SICUREZZA ALIMENTARE

| ORARIO | PRESENTAZIONI | DOCENTI |
|--------------------------------------|--|----------------------------|
| <i>Moderatore: Francesco Pomilio</i> | | |
| 9.00 - 9.10 | Apertura dei lavori e indirizzo di benvenuto | G. Migliorati, IZSAM |
| 9.10 - 9.40 | EFSA One Health WGS system and portal | M. Rossi, EFSA |
| 9.40 – 10.00 | GENPAT, piattaforma nazionale per la raccolta e conservazione delle sequenze genomiche di microrganismi patogeni: possibilità di condivisione e analisi dei dati da laboratori esterni | A. Di Pasquale, IZSAM |
| 10.00 – 10.25 | Analisi di sequenze genomiche durante un focolaio: pro e contro delle diverse metodologie. | A. Chiaverini, IZSAM |
| 10.25 – 10.50 | Genomica applicata allo studio di persistenza di <i>Listeria monocytogenes</i> negli ambienti di produzione | G. Blasi, IZSUM |
| 10.50 – 11.10 | Non solo Europa: caratterizzazione di ceppi di <i>Listeria monocytogenes</i> in prodotti ready to eat <u>zambesi</u> | G. Centorotola, IZSAM |
| 11.10 – 11.30 | Caratterizzazione di specie di <i>Listeria spp.</i> mediante MALDI TOF: verifica del metodo | M. De Angelis, IZSAM |
| 11.30 – 11.45 | Aggiornamenti dal Workshop EURL 2022 e circuiti <u>Interlaboratorio</u> | F. Pomilio, IZSAM |
| 11.45 - 11.55 | Discussione finale | Tutti i relatori coinvolti |

National reference laboratories shall, in their area of competence:

- e) provide within the scope of their mission scientific and technical assistance to the competent authorities for the implementation of multi-annual national control plans and of coordinated control programmes establishing the prevalence of certain hazards across the Union;
- f) validate the reagents and lots of reagents, establish and maintain up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents;
- g) conduct training courses for the staff of official laboratories;

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DELL'ABRUZZO
E DEL MOLISE
"G. CAPORALE"

Attività di caratterizzazione e di analisi genomica dei ceppi e raw reads prodotti nell'ambito delle indagini epidemiologiche svolte in seguito alla notifica RASFF 563256 del 02 agosto 2022. Secondo rapporto.

National reference laboratories shall, in their area of competence:

- h) assist actively the Member State having designated them in the diagnosis of outbreaks of foodborne, zoonotic or animal diseases or of pests of plants and in case of non-compliance of consignments, by carrying out confirmatory diagnoses, characterization and epizootic or taxonomic studies on pathogen isolates or pest specimens.

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/
NATIONAL REFERENCE
LABORATORY FOR
LISTERIA
MONOCYTOGENES
SETTORE 11-PRIL L.monocytogenes

European Union Reference
Laboratory for *Listeria*
monocytogenes (EURL Lm)
Maison Afort Laboratory for Food
Safety,
14, rue Pierre et Marie Curie 94701
Maisons-Afort, France
eurl.listeria@anses.fr

SUBJECT: Urgent Inquiry (UI) – New clinical cases and food isolates of *Listeria monocytogenes* CC6 S16 in the United Kingdom related to frozen vegetable outbreak.

As requested by your mail dated September 23rd, 2021, Lm strains matching the reference genome of the frozen vegetable outbreak strain were searched in our National database. Genomic comparison was performed using core genome MLST by chewBACA software (Pastour scheme).

As detailed in the attached file (Figure 1), 6 strains were found, in our database, isolated from 2 samples of frozen corn in 2018, closely related (< 7 alleles) with the outbreak strain.

Additionally, as requested, core genome MLST was performed with CC6 strains of non-human origin from vegetables and processing plants.

As detailed in the attached file (Figure 2), except for the former strains from frozen corn, no isolates showed an allelic distance < 7 alleles.

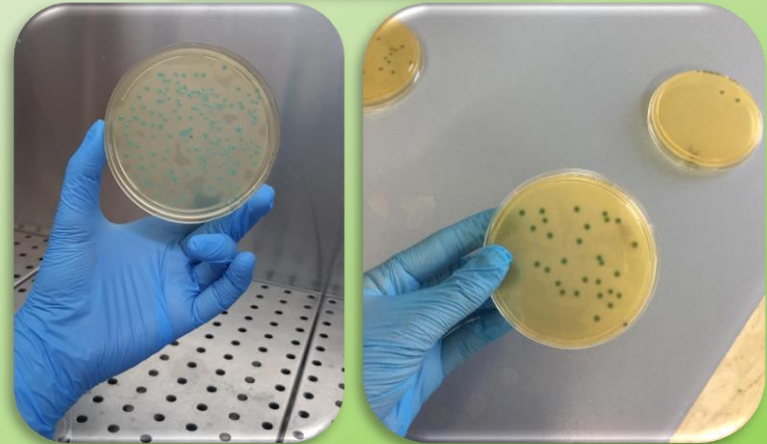
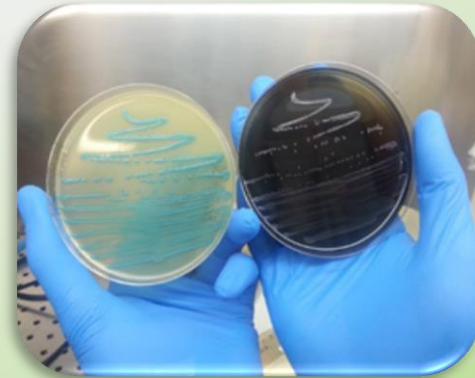
Giacomo Migliorali
SCIENTIFIC DIRECTOR

M1/vp
Enclosures: 2

Activities of Italian NRL for *Listeria monocytogenes*

Microbiological methods:

- ISO 11290-1:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method
- ISO 11290-2:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method

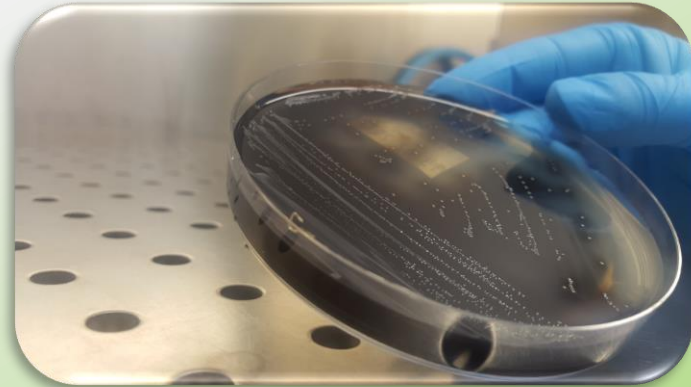


Activities of Italian NRL for *Listeria monocytogenes*

Microbiological methods:

- FSIS USDA MLG 8.13 10/01/2021 Isolation and Identification of *Listeria monocytogene* from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples

This method is applied in particular for official laboratories receiving samples from plants authorized to export to the USA meat-based products.



Immunological methods: VIDAS

- AFNOR Certification

VIDAS *L. monocytogenes* (*Lm*) is an enzyme-linked fluorescent immunoassay (ELFA). The interior of the Solid Phase Receptacle is coated with anti- *Lm* antibodies adsorbed on its surface. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

Part of the enrichment broth is dispensed into the reagent strip. The antigens present will bind to the anti- *Lm* antibodies which are coated on the interior of the SPR. Unbound sample components are washed away.

At the end of the assay, the results are analyzed automatically by the instrument which generates a test value for each sample. This value is compared to a set of stored standards (thresholds) and each result is interpreted (positive, negative).

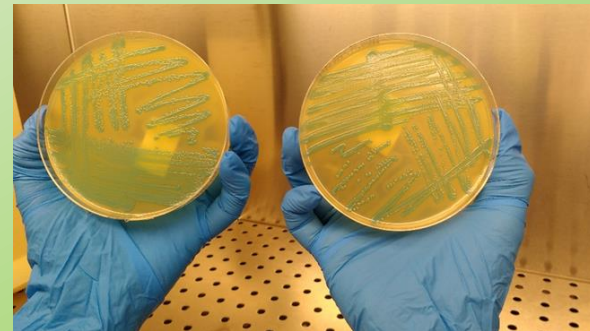
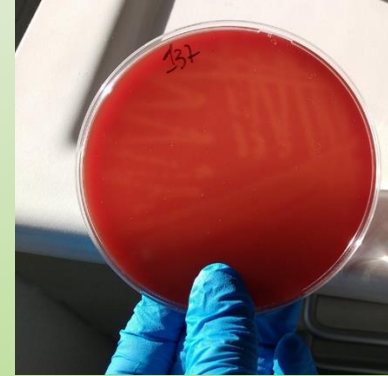
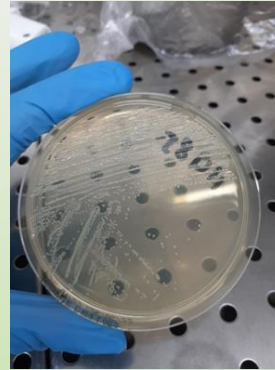


Activities of Italian NRL for *Listeria monocytogenes*

Molecular confirmation and characterization:

Starting point: typical *Listeria* colonies isolated and identified with phenotypic or molecular methods.

We usually receive from 1 to 5 colonies for each positive sample. Our aim is to confirm specie and to type it in order to understand genetic characteristics and to perform surveillance and outbreak investigation.



Confirmation and typing protocols:

Routine protocols:

- *Listeria* serogroup: multiplex PCR
- *Listeria* VIDAS
- Multilocus sequence typing (MLST)
- Core genome MLST (cgMLST)
- Virulence, resistance and stress genes detection

In silico (WGS)

Withdrawed protocol:

Listeria serotype: conventional method

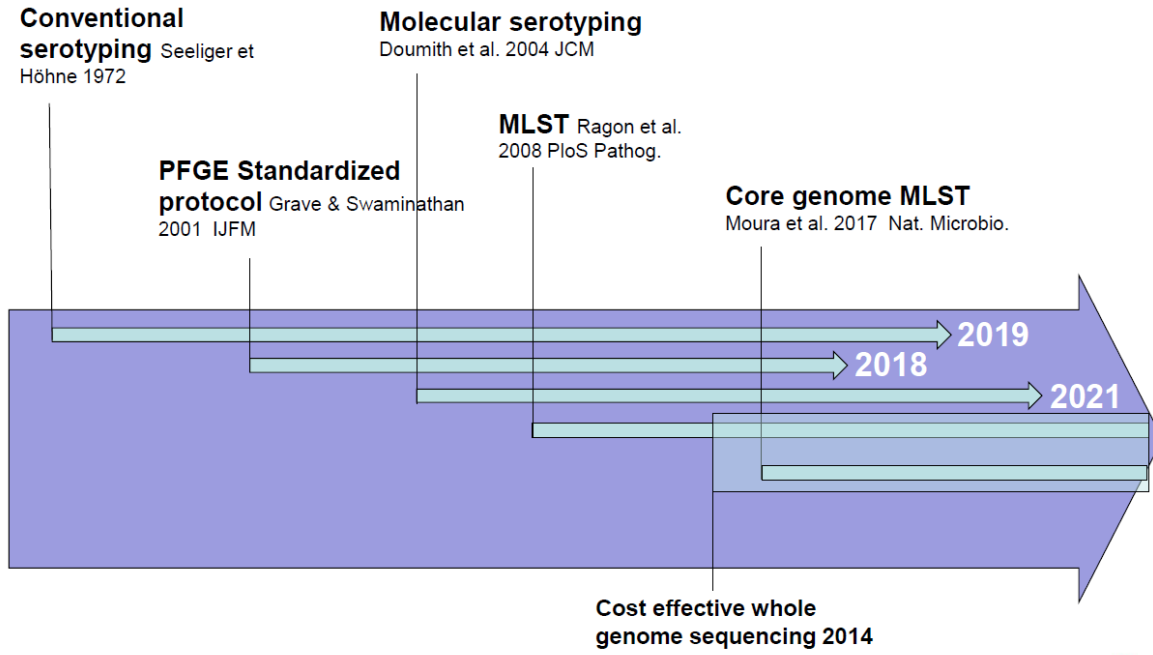
Other protocols:

- *Listeria* species: multiplex PCR
- Pulsed-field gel electrophoresis (PFGE)
- Triplex real-time PCR of 28 major clonal complexes

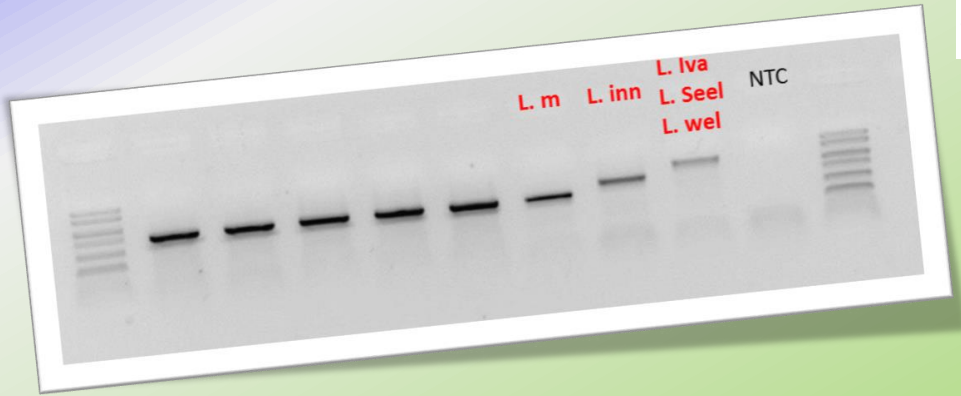
Future protocols:

- Single nucleotide polymorphism (SNP) analysis (phylogenetic studies)
- Pan genome analysis
- GWAS

History of *Lm* typing & current use at EURL *Lm*



Listeria monocytogenes species confirmation



Target: iap gene (for invasion-associated protein)
 demonstrated common and variable regions within the p60
 proteins. The variable domains appear to be specific for a
 given Listeria species.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1999, p. 4688-4692
 099-2243/99/\$04.00 + 0
 Copyright © 1999, American Society for Microbiology. All Rights Reserved.

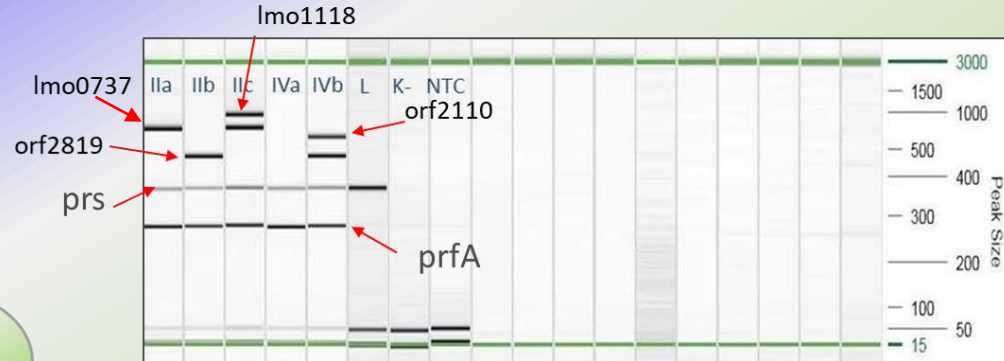
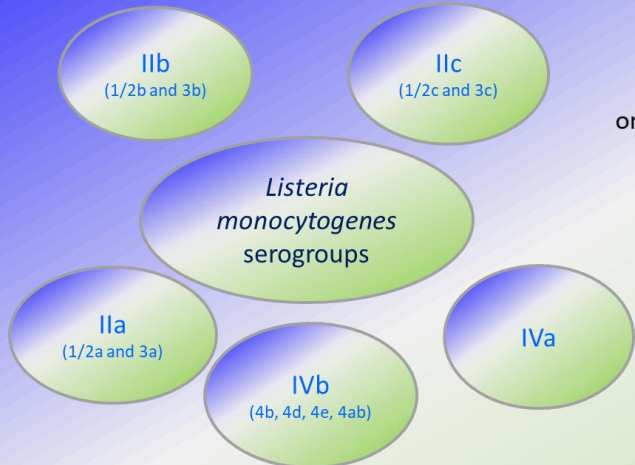
Vol. 65, No. 10

**Detection and Differentiation of *Listeria* spp. by a Single
 Reaction Based on Multiplex PCR**

ANDREAS BUBERT,^{1,2*} INGE HEIN,³ MARCUS RAUCH,¹ ANGELIKA LEHNER,³ BYOUNGSU YOON,⁴
 WERNER GOEBEL,¹ AND MARTIN WAGNER⁵

¹Lehrstuhl für Mikrobiologie, Theodor-Boveri-Institut für Biowissenschaften, Universität Würzburg, 97074 Würzburg,
 and Microbiological Analytics, Merck KGaA, 64271 Darmstadt, Germany; ²Institut für Milchhygiene und
 Milchtechnologie, Veterinärmedizinische Universität Wien, Vienna, Austria; ³and ⁴Department of Biology,
 Kyonggi University, Suwon, Kyonggi-Do 442-760, Korea⁵

Listeria monocytogenes molecular serogroup



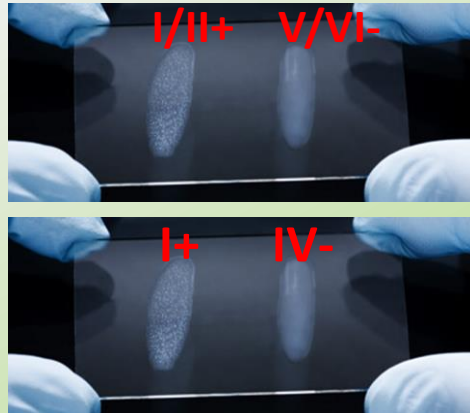
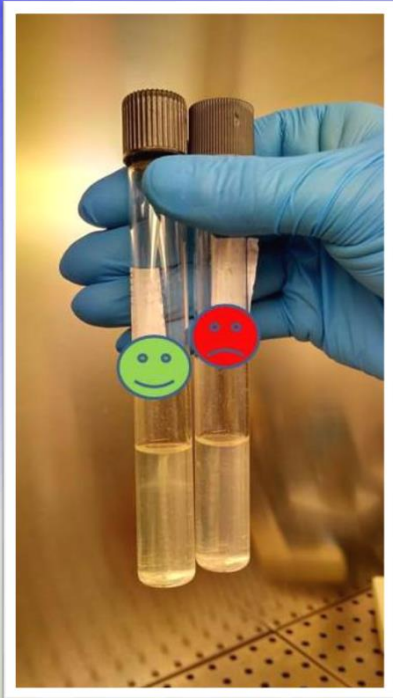
The result of the molecular group is given as indicated in the table below.

Table 4. Results expression

| Target | Expected size (or weight) | Molecular group | | | | | Listeria non-monocytogenes |
|---------|---------------------------|-----------------|-----|-----|-----|-----|----------------------------|
| | | IIa | IIb | IIc | IVa | IVb | |
| Imo1118 | (906 bp) | - | - | + | - | - | - |
| Imo0737 | (691 bp) | + | - | + | - | - | - |
| orf2110 | (597 bp) | - | - | - | - | + | - |
| orf2819 | (471 bp) | - | + | - | - | + | - |
| prs | (370 bp) | + | + | + | + | + | + |
| PrfA | (274 bp) | + | + | + | + | + | - |

Listeria monocytogenes conventional serotyping (withdrawed): O antigen

FDA Bacteriological analytical manual 8TH
 edition (Revisione A) 1998 Serodiagnosis of
Listeria monocytogenes, chap. 11, rev. 2001
[\[http://vm.cfsan.fda.gov/~ebam/bam-11.html\]](http://vm.cfsan.fda.gov/~ebam/bam-11.html)



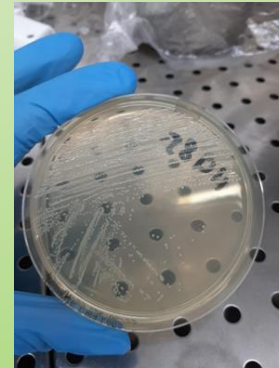
anses
 Laboratoire de sécurité des
 aliments de Maisons-Alfort

Listeria monocytogenes AGGLUTINATION SEROTYPING

Authors : MARAULT Muriel
 ROUSSEL Sophie
 Translator : Benjamin FELIX

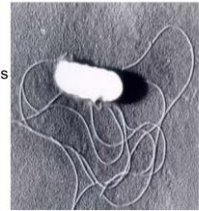
Approver: BRISABOIS Anne
original signé

Subject matter: Identification of *Listeria monocytogenes* somatic and flagellar antigen by using specific antisera.

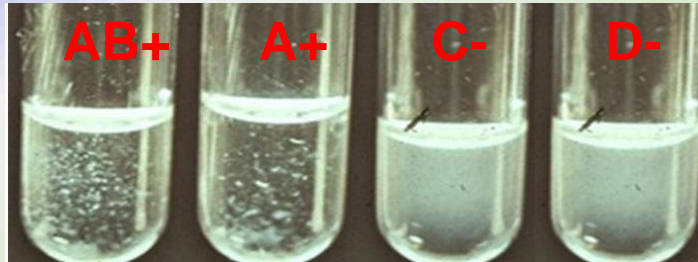
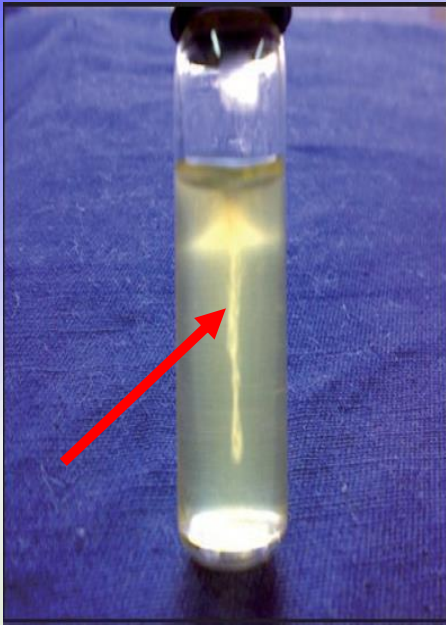


Listeria Monocytogenes

- Small cocal Gram positive Bacteria
- Occurs in chains
 - Long filamentous forms
 - Tumbling motility at 25°C and non motile at 37°C
- Peritrichous flagella
 Aerobic and Microaerophilic
 Growth at 4°C



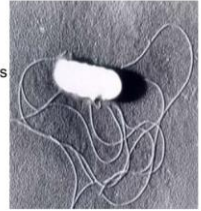
Listeria monocytogenes conventional serotyping (withdrawed): H antigen



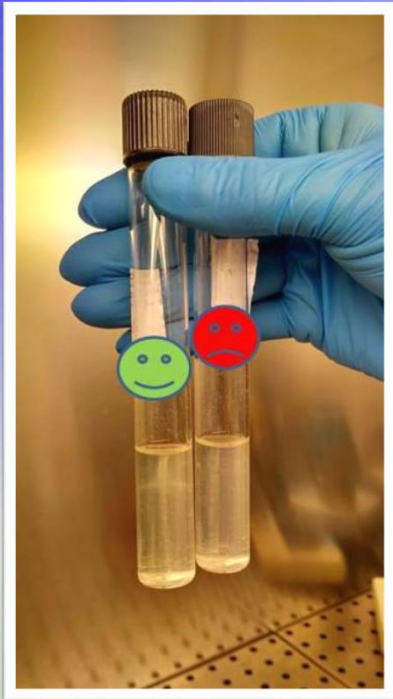
Agglutination may be seen after
incubation in waterbath for 1h
at 48°C

Listeria Monocytogenes

- Small cocal Gram positive Bacteria
- Occurs in chains
- Long filamentous forms
- Tumbling motility at 25°C and non motile at 37°C
- Peritrichous flagella
- Aerobic and Microaerophilic
- Growth at 4°C



Listeria monocytogenes conventional serotyping (withdrawed)



| Serotype | Somatic Antigen (O) | | | | | | | | | | Flagellar Antigen (H) | | | |
|----------|---------------------|---|----|------|----|-----|------|----|-----|------|-----------------------|---|---|---|
| | I/II | I | IV | V/VI | VI | VII | VIII | IX | XII | XIII | AB | A | C | D |
| 1/2 a | + | + | - | - | - | - | - | - | - | - | + | + | - | - |
| 1/2 b | + | + | - | - | - | - | - | - | - | - | + | + | + | - |
| 1/2 c | + | + | - | - | - | - | - | - | - | - | + | - | - | + |
| 3 a | + | - | + | - | - | - | - | - | - | - | + | + | - | - |
| 3 b | + | - | + | - | - | - | - | - | - | - | + | + | + | - |
| 3 c | + | - | + | - | - | - | - | - | - | - | + | - | - | + |
| 4 a | - | - | - | + | - | + | - | + | - | - | + | + | + | - |
| 4 ab | - | - | - | + | + | + | - | + | - | - | + | + | + | - |
| 4 b | - | - | - | + | + | - | - | - | - | - | + | + | + | - |
| 4 c | - | - | - | + | - | + | - | - | - | - | + | + | + | - |
| 4 d | - | - | - | + | + | - | + | - | - | - | + | + | + | - |
| 4 e | - | - | - | + | + | - | + | + | - | - | + | + | + | - |
| 7 | - | - | - | - | - | - | - | - | + | + | + | + | + | - |

FDA Bacteriological analytical manual 8TH edition (Revisione A) 1998 Serodiagnosis of *Listeria monocytogenes*, chap. 11, rev. 2001 [<http://vm.cfsan.fda.gov/~ebam/bam-11.html>]

Listeria monocytogenes Pulsed-field gel electrophoresis (PFGE)

Bacterial plate



Plugs



Lysis



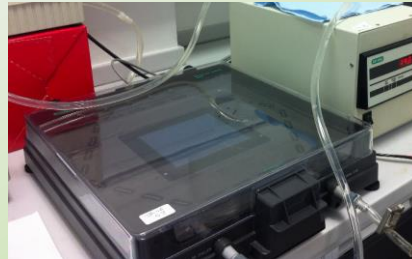
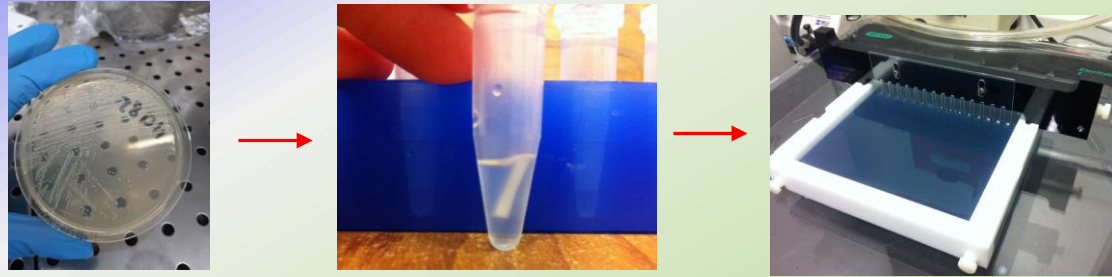
Enzymatic digestion
(*AscI/ApaI*)



Electrophoretic gel and run

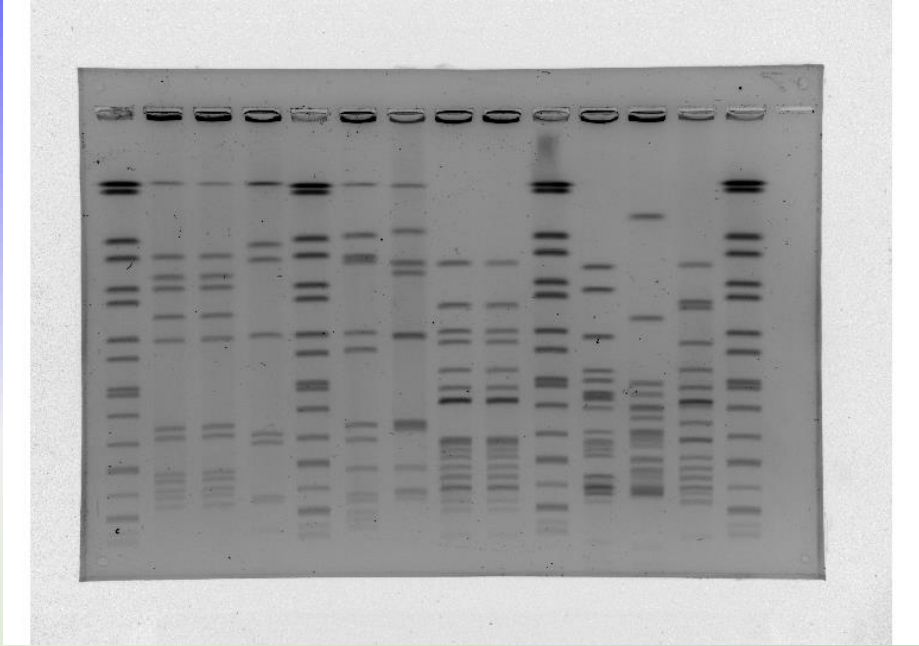


Imaging and analysis





US/CDC PNL04 Last Updated July 2017. Standard Operating Procedure
for PulseNet PFGE of *Listeria monocytogenes*.
<http://www.cdc.gov/pulsenet/PDF/listeria-pfge-protocol-508c.pdf>

Listeria monocytogenes Pulsed-field gel electrophoresis (PFGE)



US/CDC PNL04 Last Updated July 2017. Standard Operating Procedure for PulseNet PFGE of Listeria monocytogenes.
<http://www.cdc.gov/pulsenet/PDF/listeria-pfge-protocol-508c.pdf>

Real time PCR for the major Clonal Complex of *Listeria monocytogenes*



**EURL for
Lm**
European Union Reference
Laboratory for
Listeria monocytogenes

**Development of a Real Time PCR method for *Listeria
monocytogenes* frontline characterisation**
Project Geno-Listeria
Working group meeting 05/11/2020

ANSES Laboratoire de sécurité des aliments, Unité SEL, LRUE
Lm, Maisons-Alfort, France

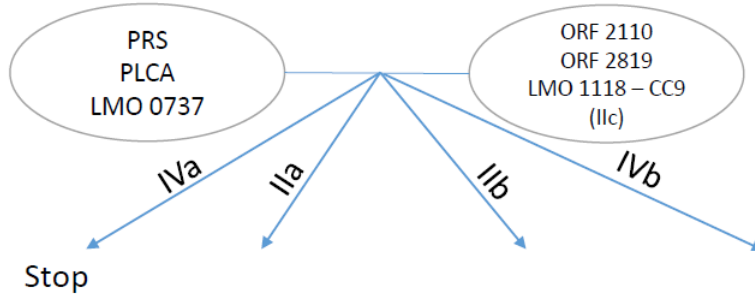
Benjamin Félix

Aims of the protocol:

- Identify the major European food CCs
- Develop a cost effective, rapid and discriminatory method
- Make the method compatible with conventional and high throughput real time PCR systems
- Validation and accreditation against the ISO16140 standard

Real time PCR for the major Clonal Complex of *Listeria monocytogenes*

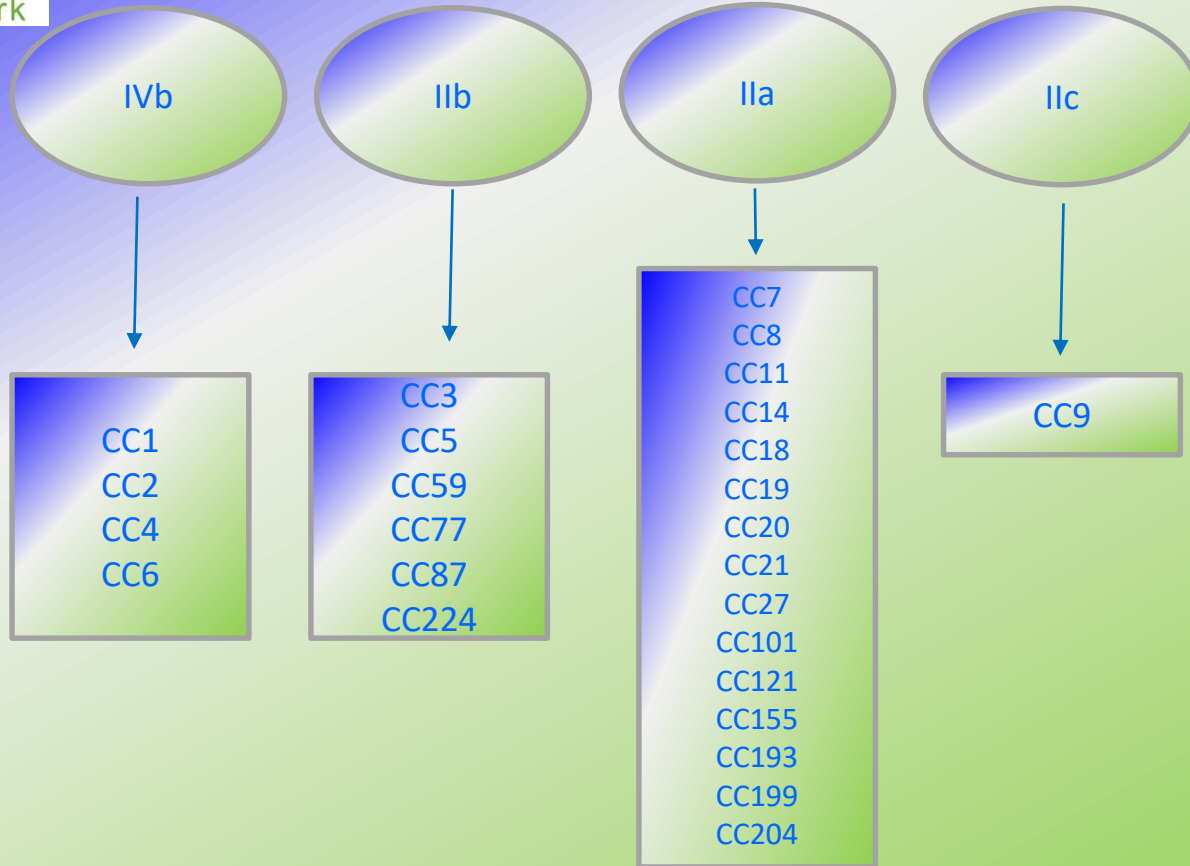
Geno*Listeria* triplex evolution: include molecular serotyping



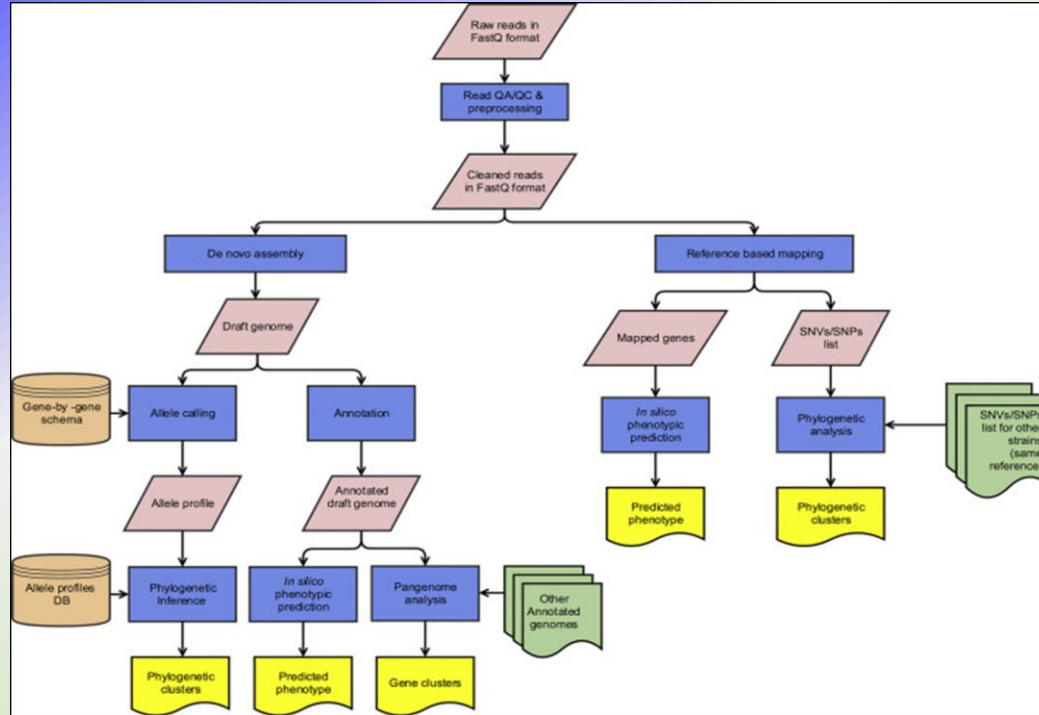
| | LMO 1118 | ORF 2110 | LMO 0737 | ORF 2819 |
|-----|----------|----------|----------|----------|
| IIa | - | - | + | - |
| IIb | - | - | - | + |
| IIc | + | - | + | - |
| IVa | - | - | - | - |
| IVb | - | + | - | + |

The first screening step involves two triplex RT PCR able to confirm the genus (*prs*), the specie (*plcA*) and the serogroup (*Lmo0737*, *Lmo1118*, *Orf2110* and *Orf2819*).

Concordance serogroup- MLST CC



In silico analysis: Whole Genome Sequencing (WGS)



Clinical Microbiology and Infection 24 (2018) 342–349

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

ELSEVIER **CMI** CLINICAL MICROBIOLOGY AND INFECTION

Review

A primer on microbial bioinformatics for nonbioinformaticians

J.A. Carrico^{1,*,} M. Rossi^{2,} J. Moran-Gilad^{3,4,5,} G. Van Domselaar^{6,7,} M. Ramirez¹

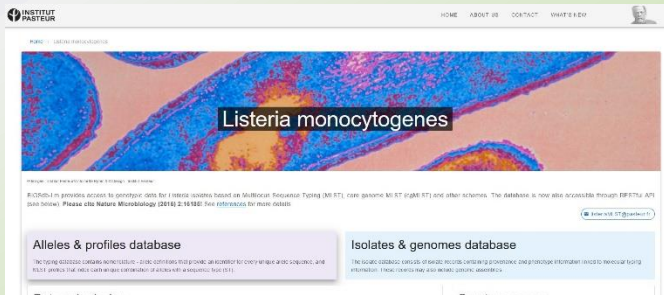
¹ Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
² Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
³ Department of Health System Management, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
⁴ Public Health Services, Ministry of Health, Jerusalem, Israel
⁵ ESCMO Study Group for Genomic and Molecular Diagnostics (ESCMO), Basel, Switzerland
⁶ National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St, Winnipeg, MB, R3E 3R2, Canada
⁷ Department of Medical Microbiology and Infectious Diseases, University of Manitoba, 743 Burnside Avenue, Winnipeg, MB, R3H 0P1, Canada

Multilocus sequence typing (ST) and Clonal complex (CC): in silico analysis

Multilocus sequence typing (MLST) is a technique in molecular biology for the typing of multiple loci, using DNA sequences of internal fragments of multiple housekeeping genes to characterize isolates of microbial species.

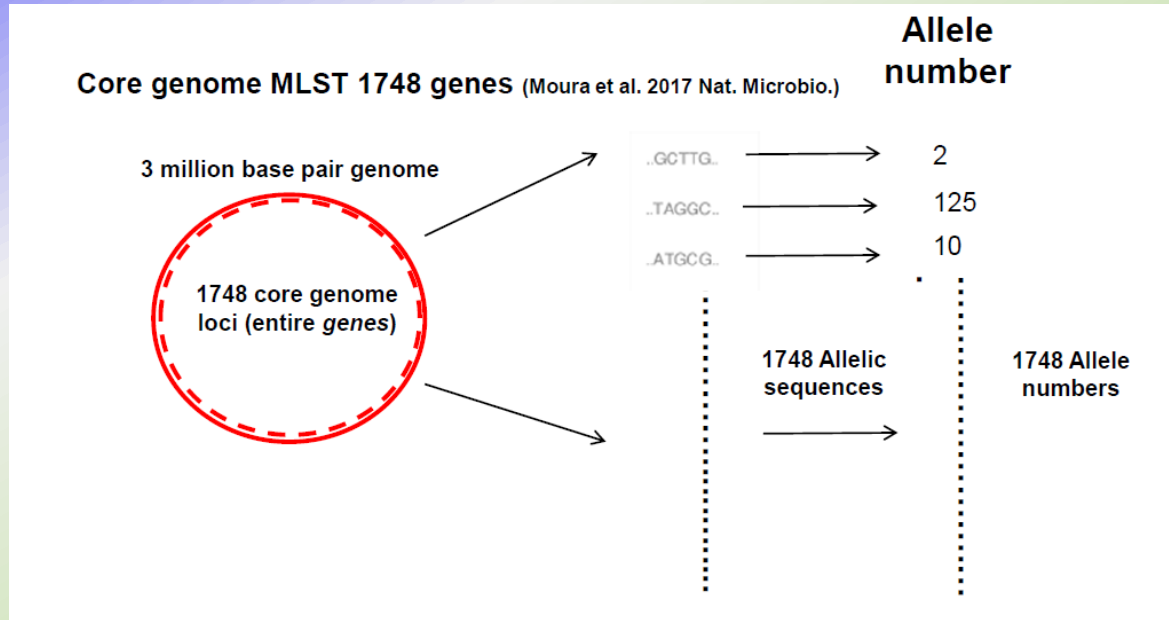
Clonal complexes were defined based on MLST data as groups of allelic profiles sharing 6 out of 7 genes with at least one other member of the group.

| ST | abcZ | bglA | cat | dapE | dat | ldh | lhxA | CC | Lineage |
|----|------|------|-----|------|-----|-----|------|-------|---------|
| 1 | 3 | 1 | 1 | 1 | 3 | 1 | 3 | CC1 | I |
| 2 | 1 | 1 | 11 | 11 | 2 | 1 | 5 | CC2 | I |
| 3 | 4 | 4 | 4 | 3 | 2 | 1 | 5 | CC3 | I |
| 4 | 1 | 2 | 12 | 3 | 2 | 5 | 3 | CC4 | I |
| 5 | 2 | 1 | 11 | 3 | 3 | 1 | 7 | CC5 | I |
| 6 | 3 | 9 | 9 | 3 | 3 | 1 | 5 | CC6 | I |
| 7 | 5 | 8 | 5 | 7 | 6 | 2 | 1 | CC7 | II |
| 8 | 5 | 6 | 2 | 9 | 5 | 3 | 1 | CC8 | II |
| 9 | 6 | 5 | 6 | 4 | 1 | 4 | 1 | CC9 | II |
| 10 | 3 | 1 | 20 | 1 | 3 | 1 | 3 | CC1 | I |
| 11 | 7 | 6 | 10 | 6 | 1 | 2 | 1 | CC11 | II |
| 12 | 5 | 8 | 5 | 7 | 6 | 22 | 1 | CC7 | II |
| 13 | 7 | 6 | 17 | 6 | 10 | 8 | 1 | ST13 | II |
| 14 | 8 | 6 | 13 | 6 | 5 | 2 | 1 | CC14 | II |
| 15 | 8 | 13 | 13 | 6 | 5 | 2 | 1 | CC14 | II |
| 16 | 5 | 6 | 2 | 7 | 5 | 2 | 1 | CC8 | II |
| 17 | 14 | 6 | 2 | 7 | 5 | 2 | 1 | CC8 | II |
| 18 | 7 | 6 | 15 | 18 | 12 | 6 | 1 | CC18 | II |
| 19 | 7 | 6 | 19 | 6 | 1 | 24 | 1 | CC19 | II |
| 20 | 17 | 13 | 3 | 6 | 5 | 7 | 1 | CC20 | II |
| 21 | 7 | 7 | 3 | 10 | 5 | 6 | 1 | CC21 | II |
| 22 | 7 | 7 | 3 | 10 | 5 | 13 | 1 | CC21 | II |
| 23 | 5 | 8 | 5 | 7 | 6 | 25 | 1 | CC7 | II |
| 24 | 5 | 8 | 5 | 22 | 6 | 2 | 1 | CC7 | II |
| 25 | 7 | 6 | 8 | 8 | 6 | 278 | 1 | CC121 | II |
| 26 | 5 | 10 | 8 | 21 | 6 | 2 | 1 | CC26 | II |
| 27 | 5 | 10 | 8 | 21 | 6 | 16 | 1 | CC26 | II |
| 28 | 104 | 6 | 8 | 8 | 6 | 37 | 1 | CC121 | II |
| 29 | 15 | 10 | 18 | 18 | 1 | 3 | 1 | CC29 | II |
| 30 | 5 | 6 | 2 | 9 | 81 | 3 | 1 | CC8 | II |

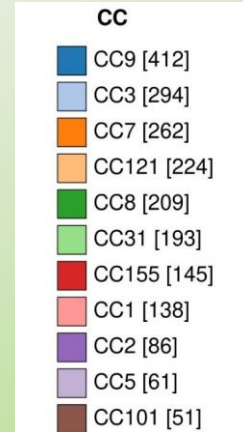
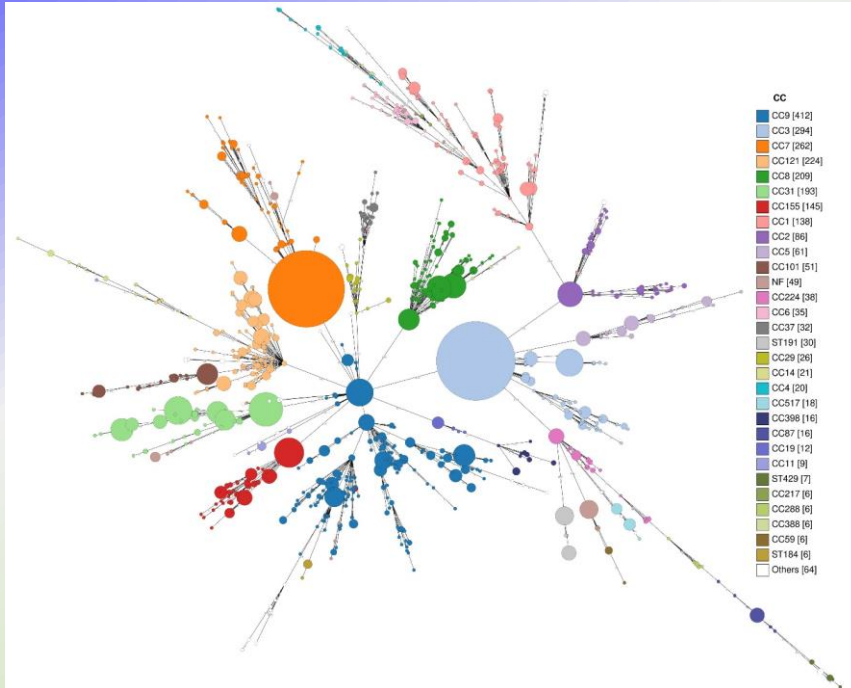


<https://bigsdB.pasteur.fr/listeria/>

Core Genome Multilocus sequence typing analysis: cgMLST

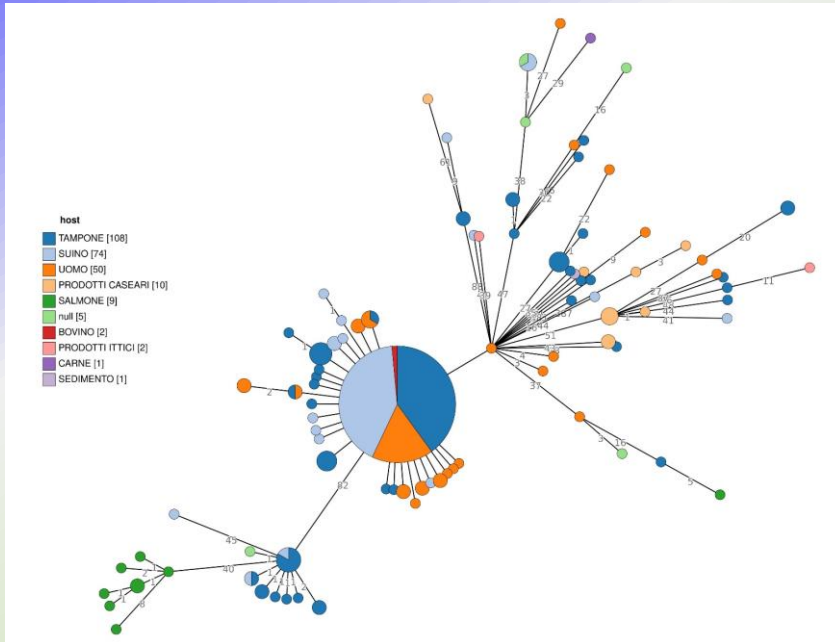


Core Genome Multilocus sequence typing analysis: cgMLST



cgMLST: minimum spanning
 tree coloured according to
 clonal complexes

Core Genome Multilocus sequence typing analysis: cgMLST



cgMLST: minimum spanning
tree coloured according to
matrices



Visualization can be done
according to different
metadata (sampling date or
place, pathogen host,
country etc...)

In silico analysis: virulence and resistance genes

STRESS SURVIVAL ISLAND

| | A | D | E | F | G | H | I | J | K |
|---|--------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | lmo1799 | l | new#1 | new#2 | new#3 | l | new#1 | new#2 | new#3 |
| 2 | lmo1800 | new#1 | 19 | new#1 | new#2 | new#1 | 19 | new#1 | new#2 |
| 3 | SSI1_lmo0444 | X | X | X | X | X | X | X | X |
| 4 | SSI1_lmo0445 | X | X | X | X | X | X | X | X |
| 5 | SSI1_lmo0446 | X | X | X | X | X | X | X | X |
| 6 | SSI1_lmo0447 | new#1 | new#2 | new#1 | new#3 | new#1 | new#2 | new#1 | new#3 |
| 7 | SSI1_lmo0448 | X | X | X | X | X | X | X | X |
| 8 | SSI2_lin0464 | X | 75 | X | 23 | X | 75 | X | 23 |
| 9 | SSI2_lin0465 | X | 80 | X | 25 | X | 80 | X | 25 |

AMR

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|----|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | aacA4 | X | X | X | X | X | X | X | X | X | X | X | X |
| 2 | aadC | X | X | X | X | X | X | X | X | X | X | X | X |
| 3 | aadE | X | X | X | X | X | X | X | X | X | X | X | X |
| 4 | aphA | X | X | X | X | X | X | X | X | X | X | X | X |
| 5 | cat_Chl | X | X | X | X | X | X | X | X | X | X | X | X |
| 6 | dfpD | X | X | X | X | X | X | X | X | X | X | X | X |
| 7 | dfxR | X | X | X | X | X | X | X | X | X | X | X | X |
| 8 | ermB | X | X | X | X | X | X | X | X | X | X | X | X |
| 9 | ermC | X | X | X | X | X | X | X | X | X | X | X | X |
| 10 | foxA | X | X | X | X | X | X | X | X | X | X | X | X |
| 11 | foxA | 7 | 1 | 4 | 1 | 9 | 3 | 2 | 9 | X | 27 | X | 27 |
| 12 | lmo0919 | 109 | 26 | 33 | 21 | 13 | new#1 | 39 | 13 | new#1 | 149 | new#1 | 137 |
| 13 | lnaA | X | X | X | X | X | X | X | X | X | X | X | X |
| 14 | lnuG | X | X | X | X | X | X | X | X | X | X | X | X |
| 15 | mefA | X | X | X | X | X | X | X | X | X | X | X | X |
| 16 | mphA | X | X | X | X | X | X | X | X | X | X | X | X |
| 17 | mprF | new#1 | new#2 | new#1 | new#1 | new#1 | 1 | new#1 | new#1 | new#1 | new#2 | new#1 | new#1 |
| 18 | murD | X | X | X | X | X | X | X | X | X | X | X | X |
| 19 | norB | 218 | 193 | 8 | 1 | 25 | 5 | 18 | 25 | new#1 | 131 | new#1 | 74 |
| 20 | penA | X | X | X | X | X | X | X | X | X | X | X | X |
| 21 | qnrB | X | X | X | X | X | X | X | X | X | X | X | X |
| 22 | str | X | X | X | X | X | X | X | X | X | X | X | X |
| 23 | sul | 6 | 169 | 6 | 1 | 1 | 4 | 11 | 1 | new#1 | 83 | new#1 | 95 |
| 24 | tetM | X | X | X | X | X | X | X | X | X | X | X | X |
| 25 | tetS | X | X | X | X | X | X | X | X | X | X | X | X |

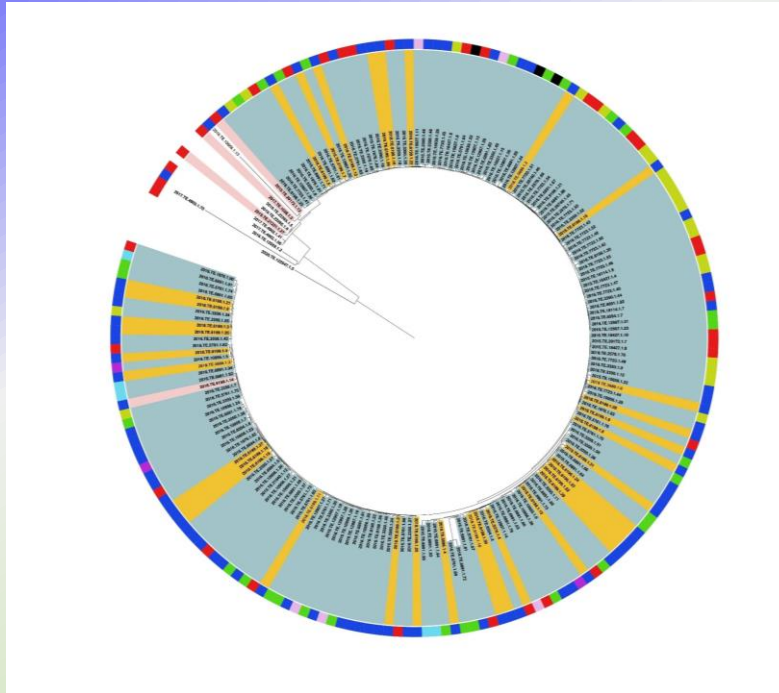
VIRULENCE

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|----|------------|-------|-------|-------|----|----|-------|-------|----|-------|-------|-------|-------|
| 1 | aha | 159 | 141 | new#1 | 17 | 10 | 133 | 10 | | | | | |
| 16 | gfb | X | X | X | X | X | X | X | X | new#1 | X | new#1 | new#1 |
| 17 | gfbA | X | 65 | X | 1 | 1 | 1 | 1 | 1 | new#1 | X | new#1 | 11 |
| 18 | hly | 15 | 15 | 17 | 2 | 16 | 11 | 117 | 11 | new#1 | X | new#1 | X |
| 19 | hnt | 7 | 171 | 9 | 10 | 20 | 1 | 21 | 20 | 6 | X | 6 | X |
| 20 | hna | 172 | 13 | 11 | 17 | 20 | 10 | 21 | 20 | new#1 | X | new#1 | X |
| 21 | hnb | 141 | 20 | 10 | 13 | 13 | 1 | 41 | 11 | X | X | X | X |
| 22 | hnc | 11 | new#1 | 8 | 7 | 11 | 7 | 13 | 11 | X | X | 6 | X |
| 23 | hnc2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | X | X | X | X |
| 24 | hnd | 49 | 10 | 13 | 42 | 13 | 8 | 14 | 11 | X | X | X | X |
| 25 | hntE | 47 | 14 | 7 | 9 | 12 | 11 | 6 | 12 | X | X | X | X |
| 26 | hntF | 1 | X | X | 1 | 9 | 1 | 1 | 1 | X | X | X | X |
| 27 | hntG | 11 | X | 1 | 4 | 4 | 2 | X | 4 | X | X | X | X |
| 28 | hntH | 24 | 13 | 1 | 8 | 10 | 1 | 35 | 10 | X | X | X | X |
| 29 | hntI | 14 | 20 | 5 | 7 | 9 | 1 | new#1 | 3 | X | X | X | X |
| 30 | hntK | 2 | 17 | 1 | 8 | 12 | 11 | 4 | 12 | new#1 | X | new#1 | X |
| 31 | hntL | X | X | X | X | X | X | X | X | X | X | X | X |
| 32 | hntP1 | X | X | X | X | X | X | X | X | X | X | X | X |
| 33 | hntP3 | X | X | X | X | X | X | X | X | X | X | X | X |
| 34 | hntP4 | X | X | X | X | X | X | X | X | X | X | X | X |
| 35 | hntPq | X | X | X | X | X | X | X | X | X | X | X | X |
| 36 | hsp | 10 | 14 | 10 | 11 | 1 | 1 | 48 | 1 | new#1 | 116 | new#1 | 118 |
| 37 | hspB | new#1 | 10 | 10 | 6 | 1 | new#1 | 41 | 1 | X | X | X | X |
| 38 | hspE | 1 | 1 | 1 | 1 | 1 | 1 | 6 | 1 | new#1 | 77 | new#1 | 81 |
| 39 | LIPI2_hnt1 | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 40 | LIPI2_hnt2 | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 41 | LIPI2_hntE | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 42 | LIPI2_hntF | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 43 | LIPI2_hntG | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 44 | LIPI2_hntH | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 45 | LIPI2_hntI | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 46 | LIPI2_hntJ | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 47 | LIPI2_hntK | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 48 | LIPI2_hntL | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 49 | LIPI2_hntM | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 50 | LIPI2_hntN | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 51 | LIPI3_hntA | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 52 | LIPI3_hntB | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 53 | LIPI3_hntC | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 54 | LIPI3_hntD | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 55 | LIPI3_hntE | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 56 | LIPI3_hntF | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 57 | LIPI3_hntG | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 58 | LIPI3_hntH | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 59 | LIPI3_hntI | 59 | 25 | 13 | 11 | 1 | 12 | 11 | 1 | new#1 | 6 | new#1 | 102 |
| 60 | LIPI3_hntJ | 10 | 14 | 1 | 7 | 1 | 1 | 3 | 1 | new#1 | new#2 | new#1 | 84 |
| 61 | LIPI3_hntK | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 62 | LIPI3_hntL | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 63 | LIPI3_hntM | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 64 | LIPI3_hntN | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 65 | LIPI3_hntO | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 66 | LIPI3_hntP | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 67 | LIPI3_hntQ | X | X | X | X | X | X | X | X | new#1 | X | new#1 | X |
| 68 | LIPI3_hntR | 140 | new#1 | 8 | 10 | 14 | 11 | 43 | 14 | X | X | X | X |
| 69 | LIPI3_hntS | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | X | X | X | X |
| 70 | LIPI3_hntT | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | X | X | X | X |

BIGSdb Genome Comparator Jolley & Maiden (2010). BMC Bioinformatics 11:595

<https://bigsdb.pasteur.fr/listeria/>

In the next future: SNP analysis



Cluster

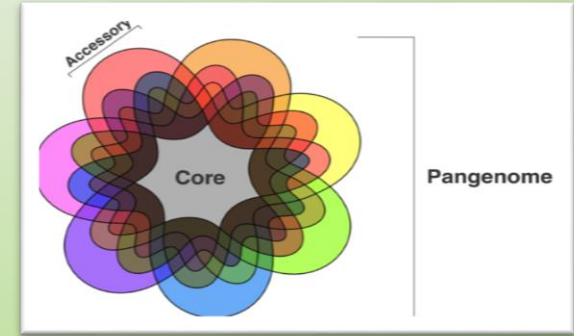
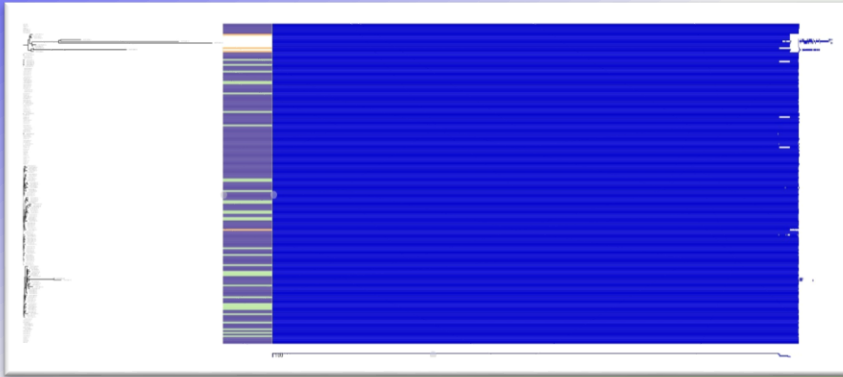
- EpiCl
- ReE
- C2

Matrix

- Clinical
- Environment
- Hog head cheese
- Salami
- Pancetta
- Bresaola
- Loin
- Pork cheek

To investigate the **phylogeny** of selected strains (e.g. outbreak strains)

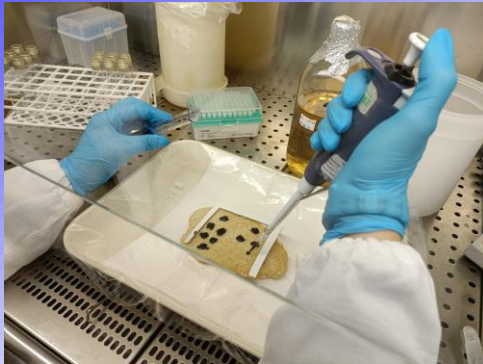
In the future: Genome wide Association Study (GWAS)



To detect significant genes associated with selected strains and/or matrices, conditions etc...

Association of genomic feature to phenotype (antimicrobial resistance, virulence factor, host variation etc.)

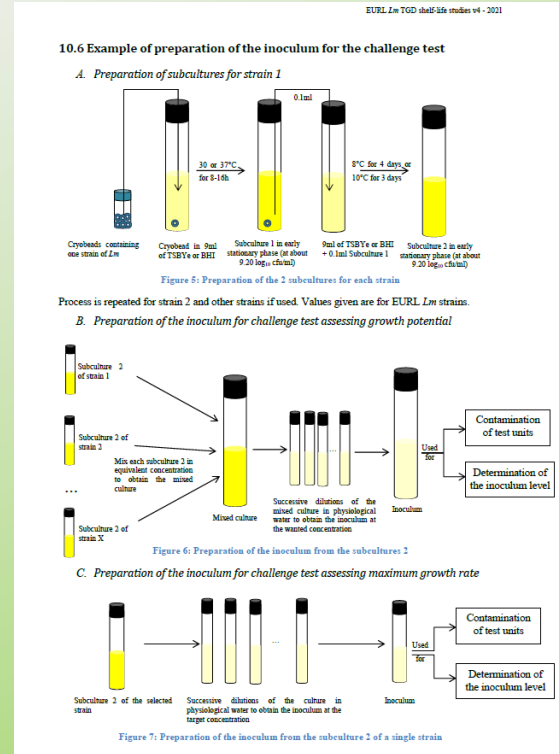
Not only genomes... challenge studies



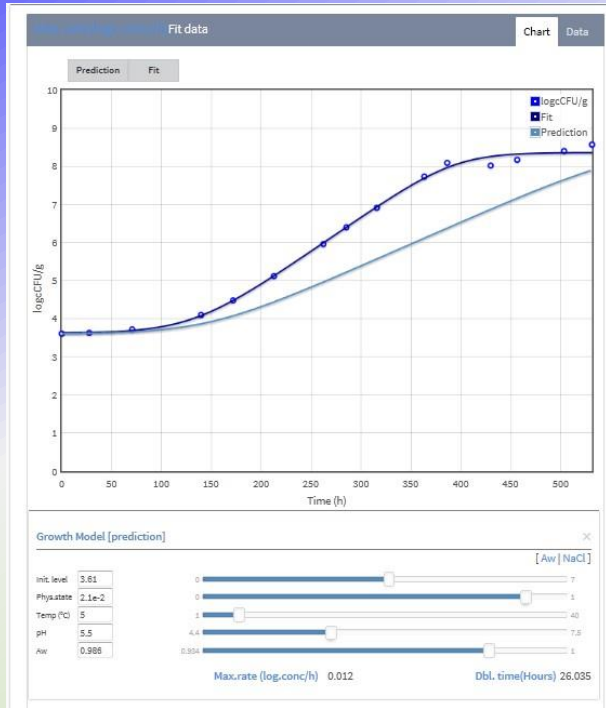
Assessing of:

- Growth potential
- Maximum growth rate

Durability studies



Not only genomes... predictive microbiology



Based on mathematical model, the software is able to describe the effects due to different parameters (pH, temperature, aw etc...) and to predict Listeria growth and survival at defined conditions



THANK YOU!

m.torresi@izs.it