# Advanced analytical methods for microbial foodborne hazards

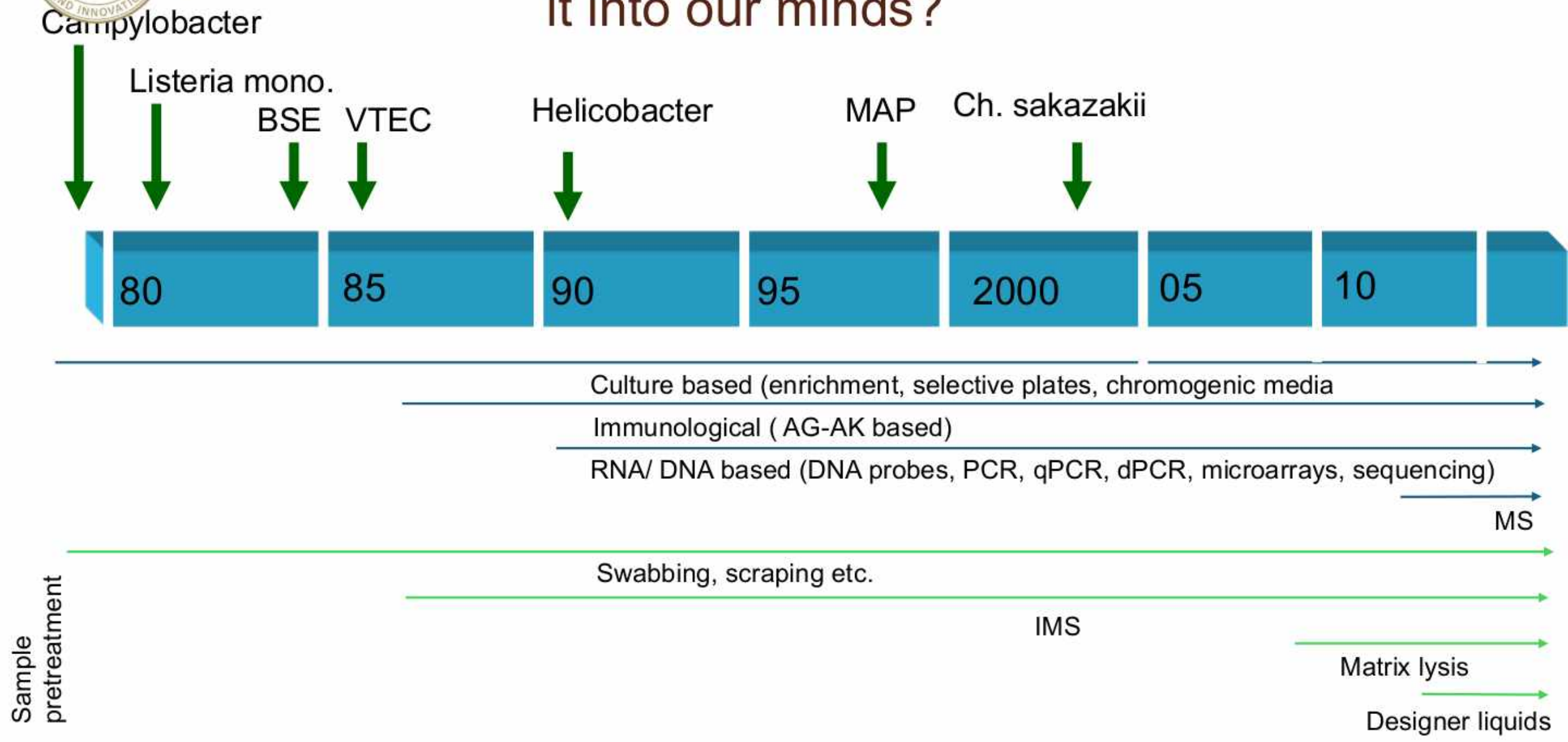
Patrick Mester, Martin Wagner and consortium

Center for Food Safety and VPH
University for Veterinary Medicine, Vienna

Austrian Competence Center for Food Quality, Safety and Innovation, Technopark 1 D, Tulln

Wageningen, 10th and 11th of June 2026.

# Major changes in technology that came it into our minds?



# Do single cells grow to populations?

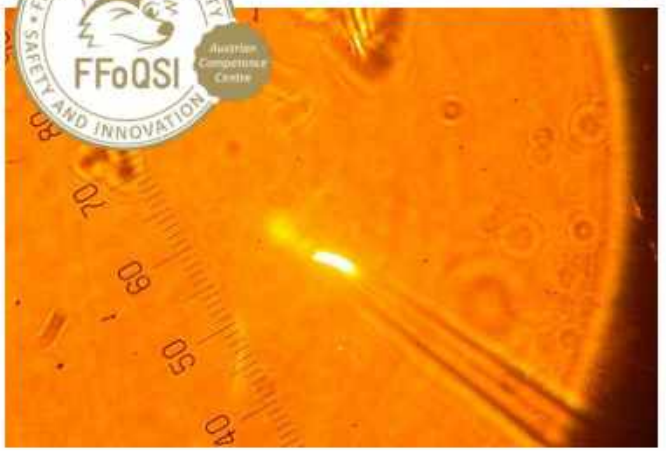
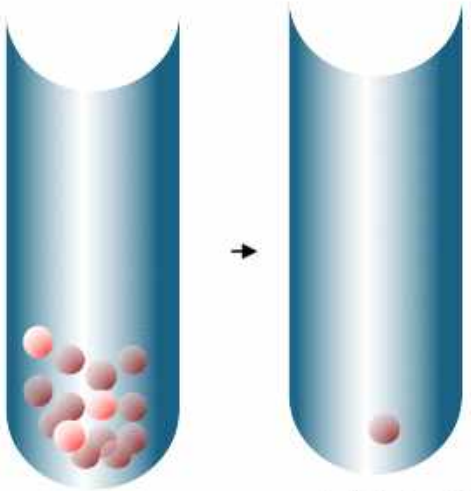


TABLE 2. Results of the single cell growth experiments[a]

| Bacterium and growth phase | % Viable cells in initial culture[b] | n[c] | % Recultivated cells (ratio) after SBCM | Final concn of recultivated cells (CFU/ml)[d] (±RSD [%]) |
|---|---|---|---|---|
| *L. monocytogenes* | | | | |
| Lag | 80 | 40 | 85 (35/40) | $1.17 \times 10^9$ (±8.8) |
| Log | 76 | 40 | 70 (28/40) | $1.21 \times 10^9$ (±7.2) |
| Stationary | 54 | 40 | 60 (24/40) | $1.25 \times 10^9$ (±11.2) |
| *S.* Typhimurium | | | | |
| Lag | 86 | 55 | 80 (44/55) | $6.80 \times 10^8$ (±8.5) |
| Log | 78 | 55 | 73 (40/55) | $7.74 \times 10^8$ (±6.9) |
| Stationary | 61 | 55 | 67 (37/55) | $7.80 \times 10^8$ (±2.6) |



TSB - Y

Live/dead ratio of the initial culture: 20.9% (± 20.6%)

24 hours, 37° C

**Positive: 69 samples** (71.5%)

**Selective plating** (PALCAM, Ocla)

**Optical density**: $1{,}21 \times 10^9$ CFU/ml (± 9.1%)

**Negative: 31 samples** (28.5%)

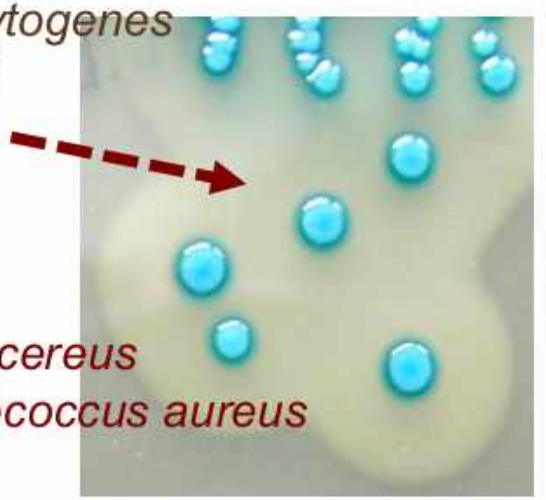
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2010, p. 2600–2606 Vol. 76, No. 8
0099-2240/10/$12.00 doi:10.1128/AEM.01506-09


Autonomous Growth of Isolated Single *Listeria monocytogenes* and *Salmonella enterica* Serovar Typhimurium Cells in the Absence of Growth Factors and Intercellular Contact[▽]

Barbara Roeder,[1] Martin Wagner,[2] and Peter Rossmanith[1]*

# Bias of culture based detection

**Phospholipase C**
*L. monocytogenes*
*L. ivanovii*

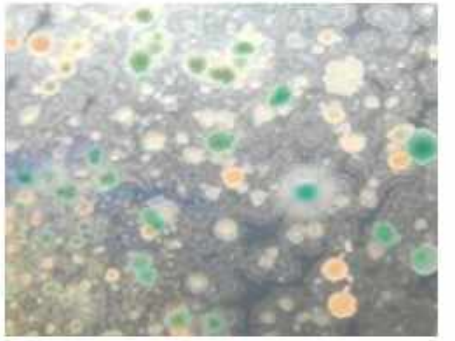


**ß-D glucosidase**
(*Listeria spp.*)

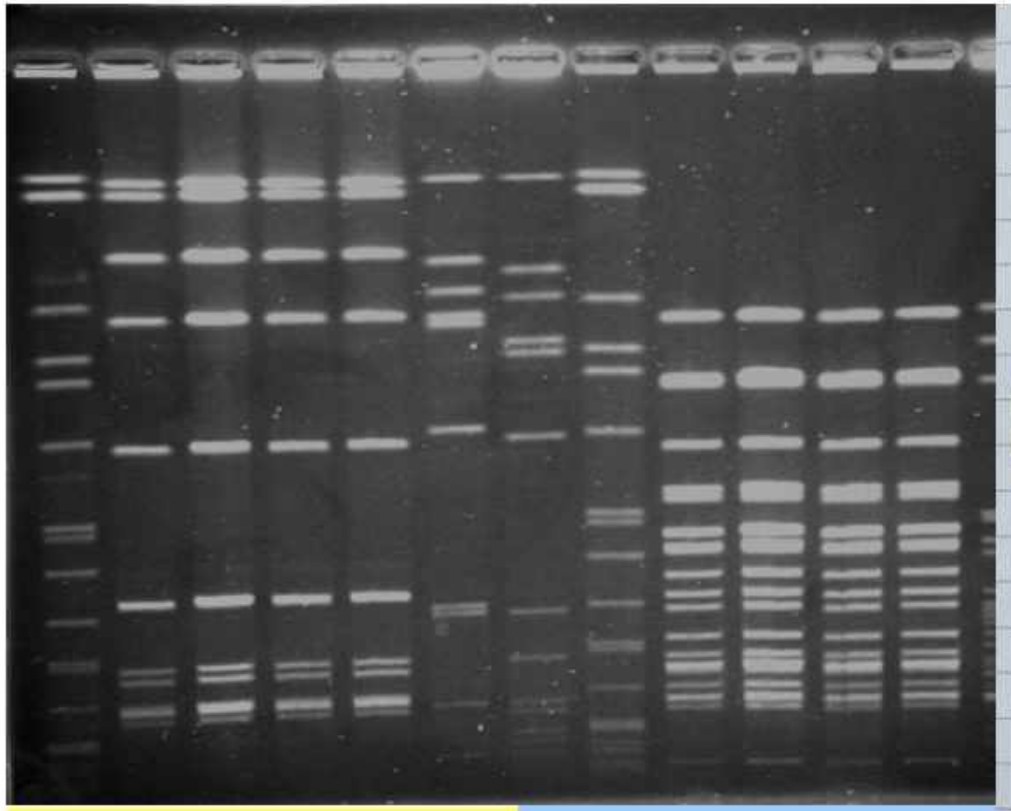
*Bacillus cereus*
*Staphylococcus aureus*

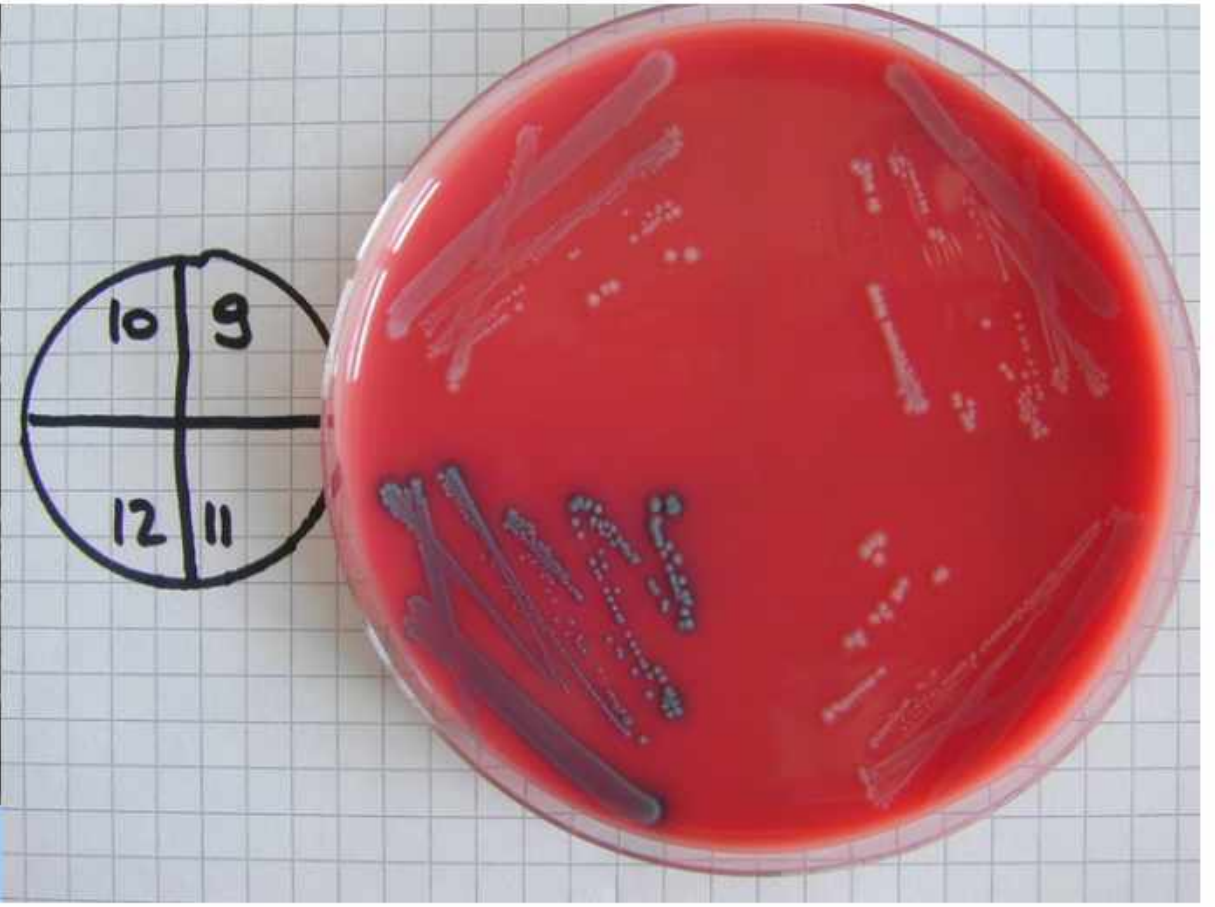
*Enterococcus spp.*
*Coryneforme*
*Bacillus circulans*



# Growth is stress...stress changes phenotype



# Nature always shows variance

- We could show that our strain harbors a different mutation in prfA
- an insertion of seven base pairs (AGTCAGG) at position 518-524 was detected in prfA (in the meanwhile more mutations detected)

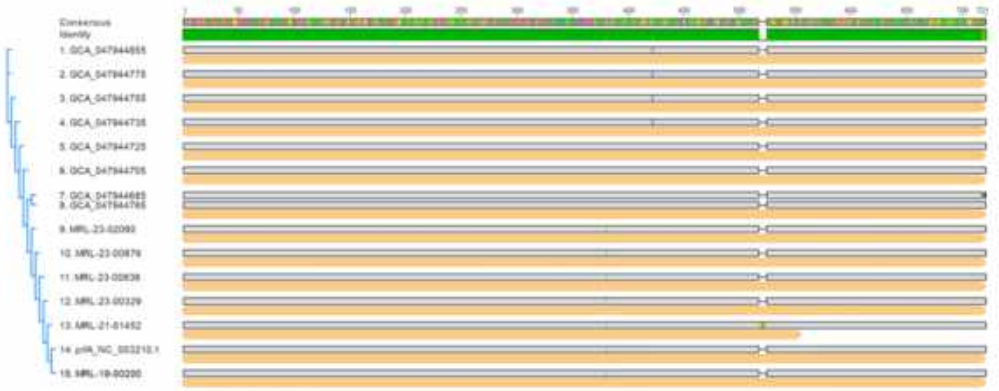
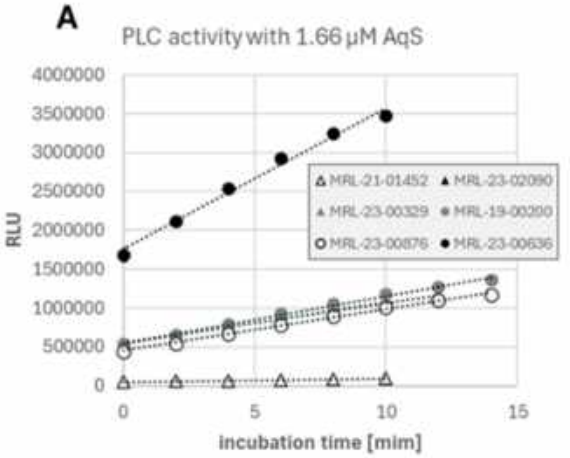


**Fig. 3.** Nucleotide alignment for *L. monocytogenes prfA*. Alignment indicates the C424G mutation only in four genomes from Stevens et al. (shown in blue). *PrfA* of MRL-21-01452 contains an insertion of seven base pairs at position 518-524. Orange bars indicate the open reading frame for each gene.




Diagnostic Microbiology & Infectious Disease 113 (2025) 117024

Contents lists available at ScienceDirect

Diagnostic Microbiology & Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Short Communication

## Risk of false-negative diagnostics of *Listeria monocytogenes* due to weak phospholipase activity

Lauren V. Alteio [a], Ariane Pietzka [b], Laura van den Höövel [c], Patrick Mikuni-Mester [c,d,*]

Applied and Environmental Microbiology, May 2011, p. 3478–3483 Vol. 77, No. 10
0099-2240/11/$12.00 doi:10.1128/AEM.02708-10

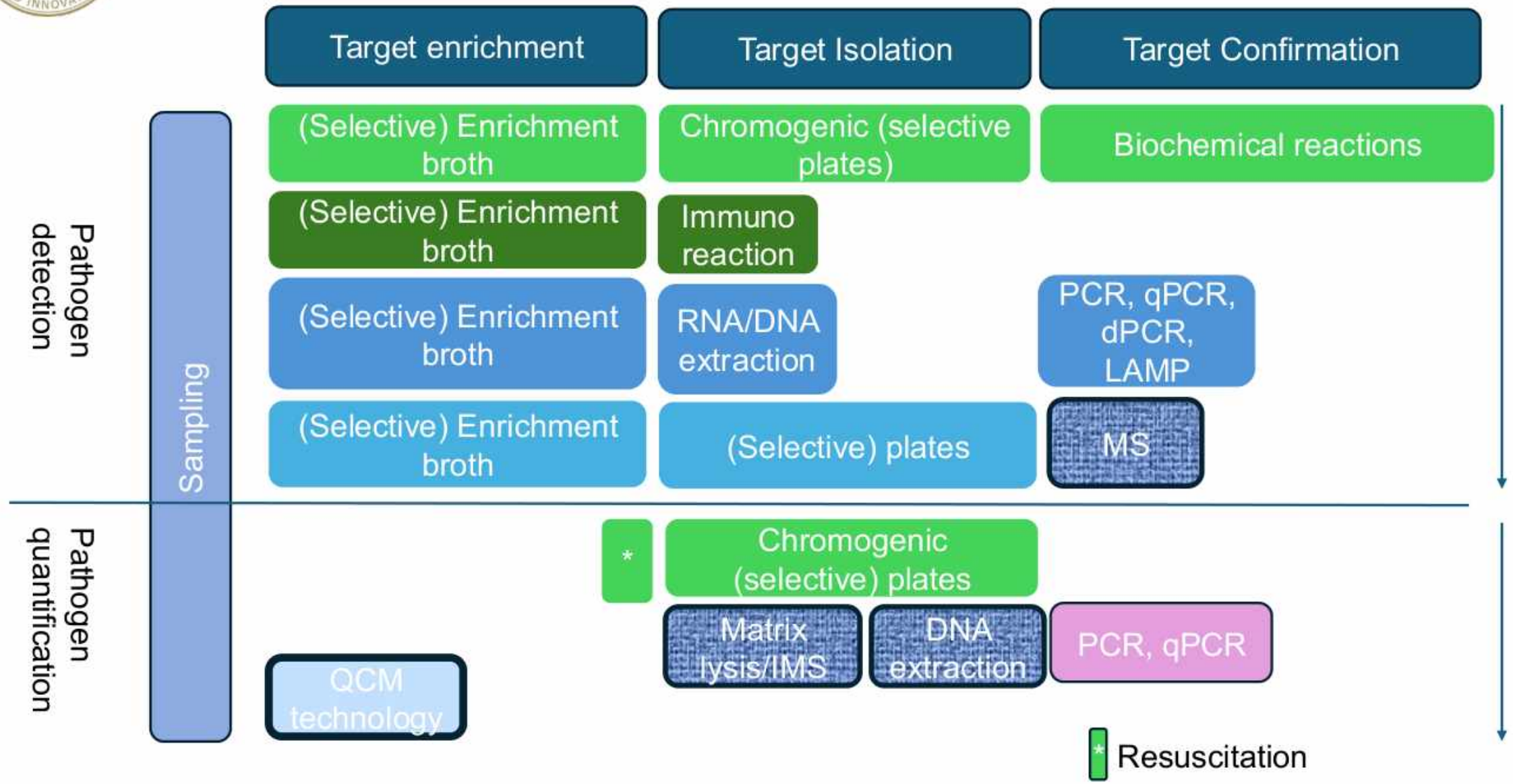

## A Contingency Locus in *prfA* in a *Listeria monocytogenes* Subgroup Allows Reactivation of the PrfA Virulence Regulator during Infection in Mice[▽]

Toril Lindbäck,* Indira Secic, and Liv Marit Rørvik

*Department of Food Safety and Infection Biology, Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N-0033 Oslo, Norway*

Received 19 November 2010/Accepted 22 March 2011

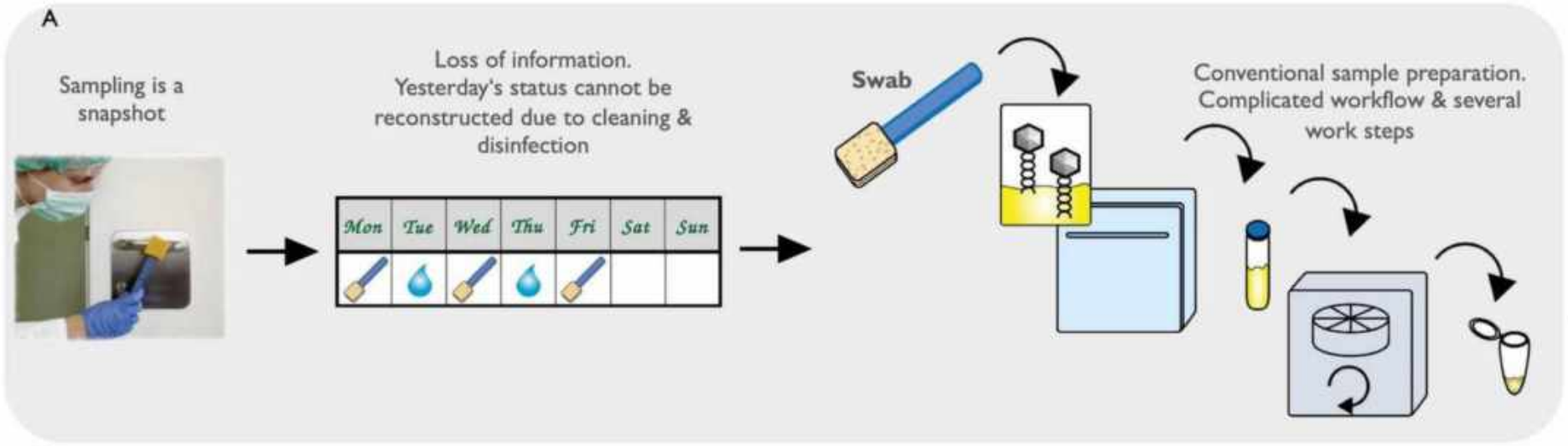
# Theoretical frame work

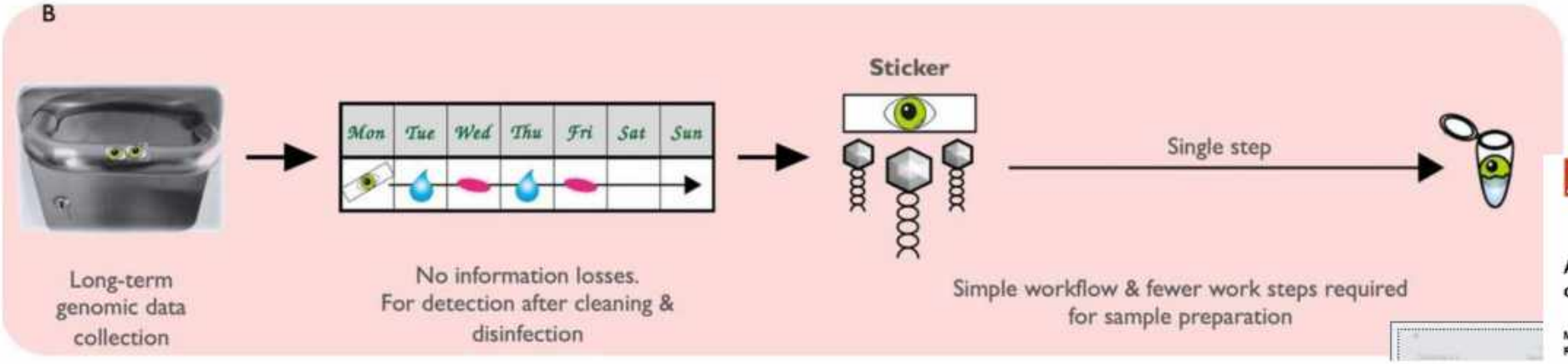


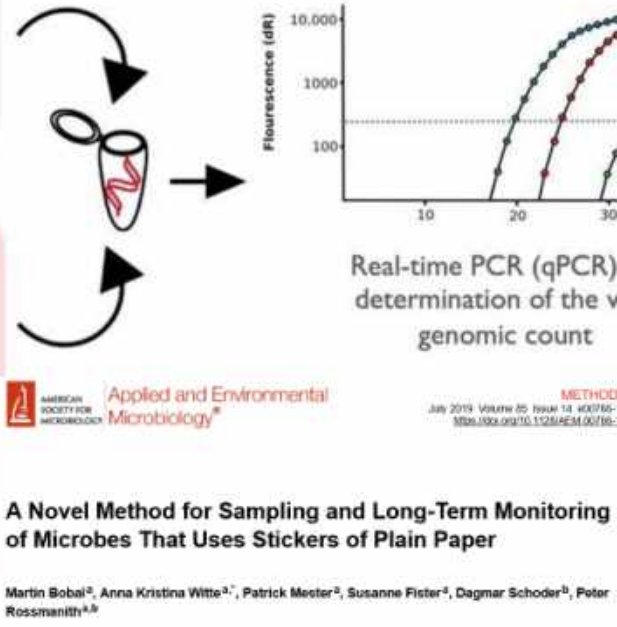
# Questions food industry asks us…

| FAQ | |
|---|---|
| Is this a pathogenic species? | We need a well established detection platform |
| Do we see repeated transmissions into the FBO? | We need typing information |
| How can we clean them away? | We need to run bioassys (FBO isolates aren´t lab strains |
| Are we safe (can we deliver)? | A result of answers to previous questions |
| What is the price? | |
| | |

# Advanced sampling by a reversed sticker approach



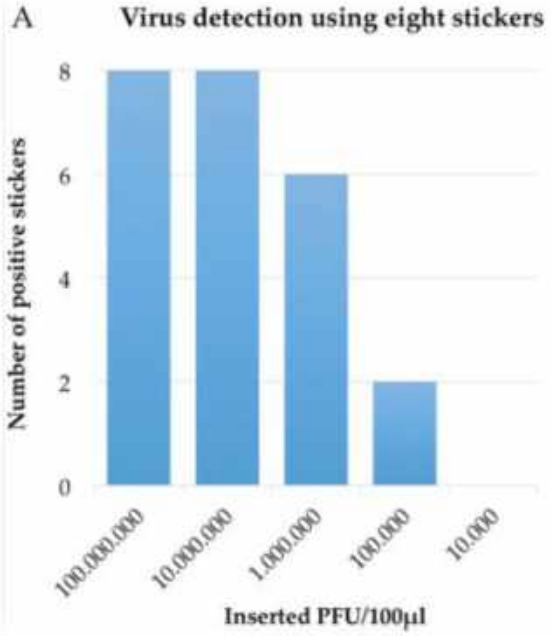



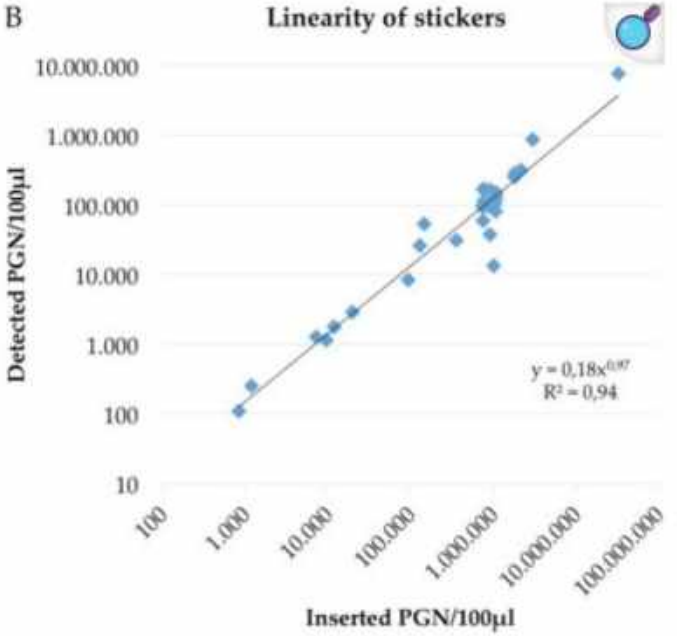



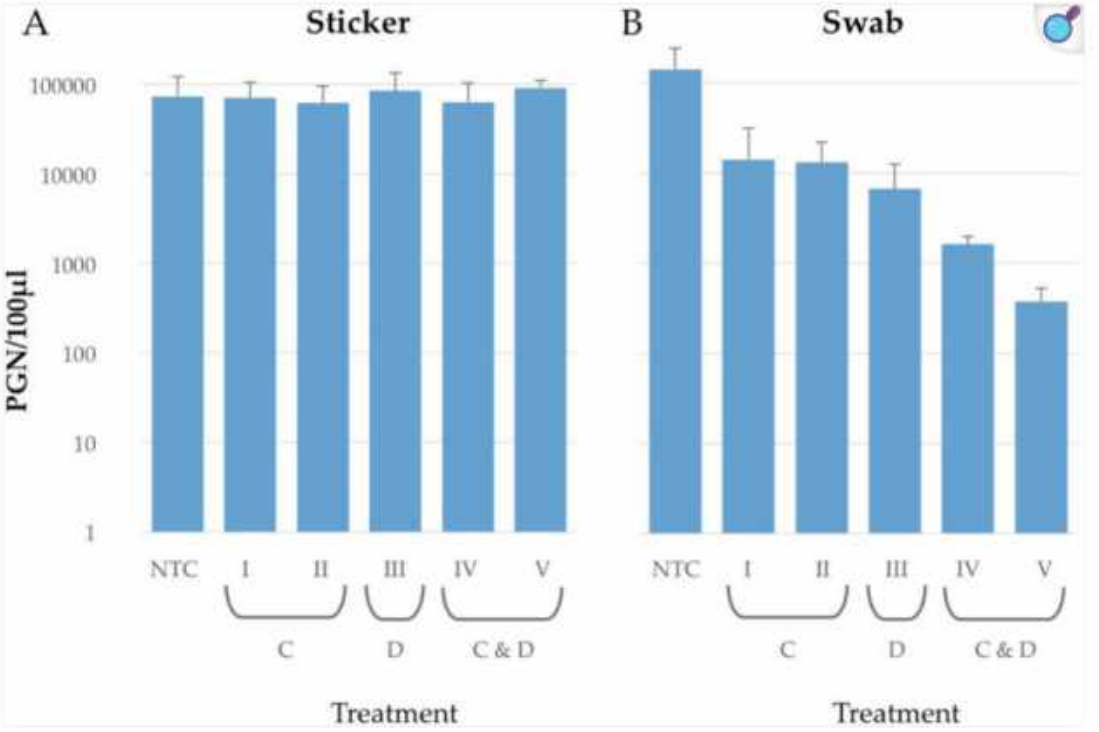

**A Novel Method for Sampling and Long-Term Monitoring of Microbes That Uses Stickers of Plain Paper**

Martin Bobal[a], Anna Kristina Witte[a,c], Patrick Mester[a], Susanne Fister[a], Dagmar Schoder[b], Peter Rossmanith[a,b]

# Advanced sampling by a reversed sticker approach




scientific reports

OPEN

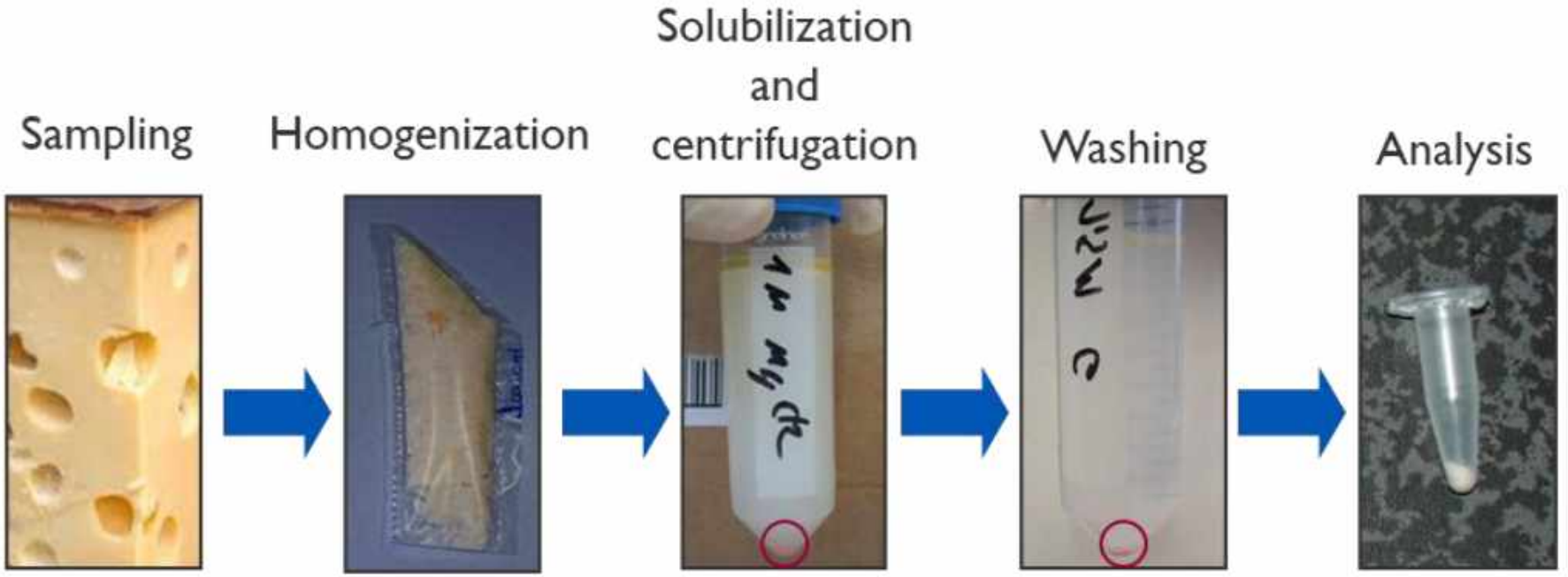
## A new long-term sampling approach to viruses on surfaces

Julia Sommer[1,2], Martin Bobal[1,3], Birgit Bromberger[1], Patrick-Julian Mester[1] & Peter Rossmanith[1,2]

# Matrix lysis

- Concept to solubilize solid food stuffs
- Number of microbial cells is not changed (a basis for molecular quantification)
- IPC designed and applied
- Cells are harvested by centrifugation

# Innovative sample preparation by Matrix lysis



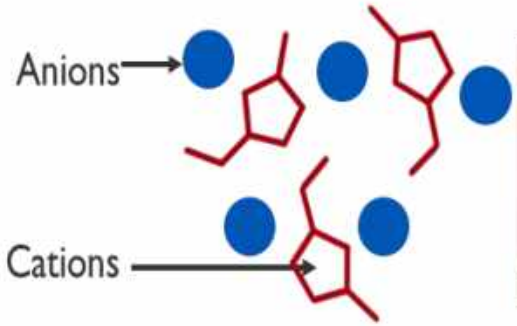

6.25g / 12.5 ml → <100µl

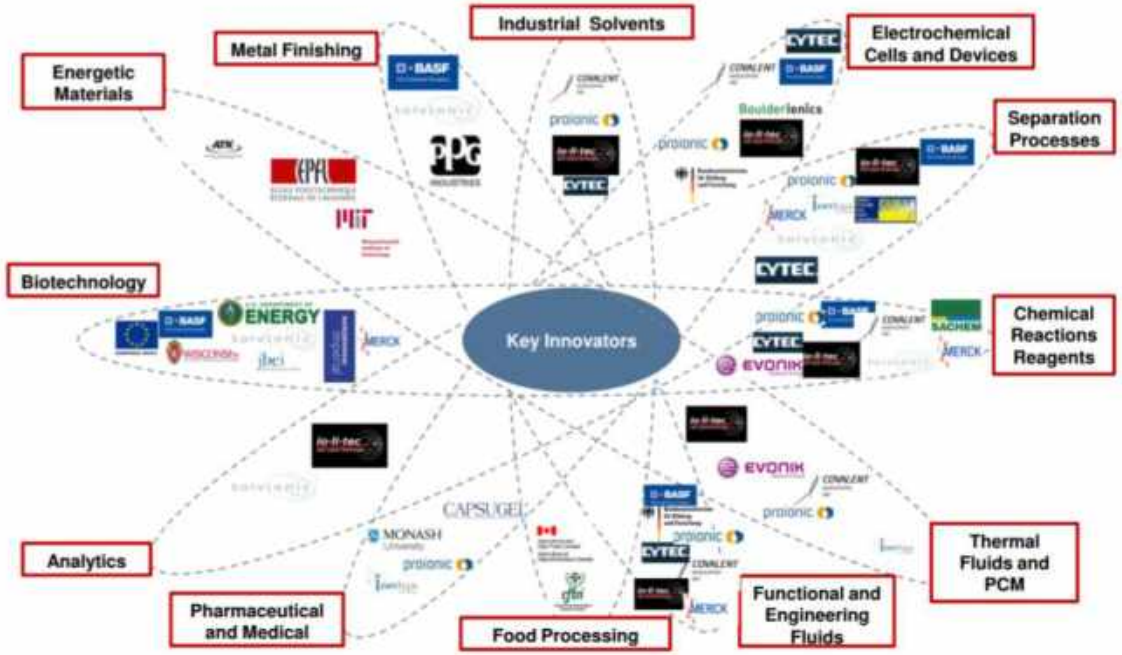
60 – 120 fold concentration

20 min -3 h duration

# Ionic liquids in food science

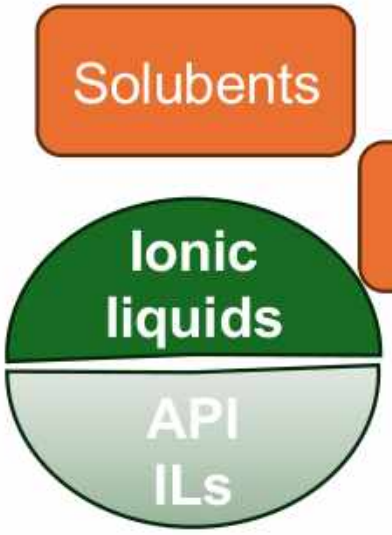
- "Ionic liquid" : entirely composed of ions and are liquid at temperatures below <100°C.
- ILs are remarkable, **highly tunable** chemical compounds with exceptional properties
- Used in a lot of technical disciplines but not used in food science before 2010



# Ionic liquids in microbial analytics

Any drug **formulated as an API-IL** can be modified in terms of their




Solubents

DNA extraction chemistry

Anti-microbials

Virucides

Physical properties

Melting point

Thermal stability

Solubility

Shamshina et al., Nature 188-189 (2015)

Chemical properties

Lipophilicity

Chemical stability

Permeability

Biological properties

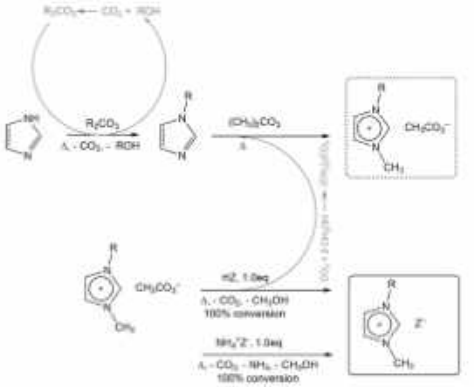
Bioavailability

Pharmacokinetics

Toxicity

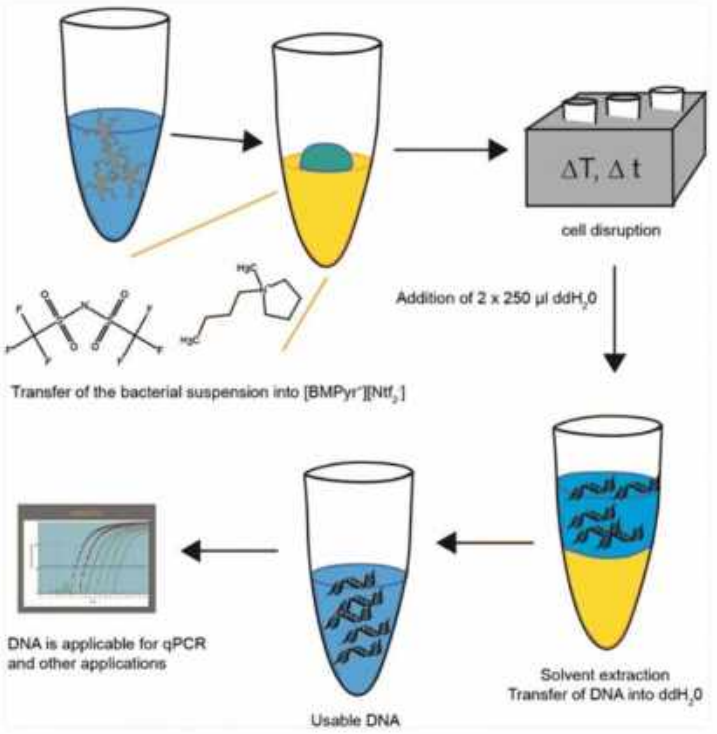
# Building a IL library at Vetmeduni

- For more than ten years we are having a strategic partnership with proionic, one of the world´s leading IL developer and producer and winner of the ***Green frontrunner award 2023***
- Utilizing the flexible, energy and resource efficient CBILs synthesis we already synthesized > 1000 unique ILs for our projects



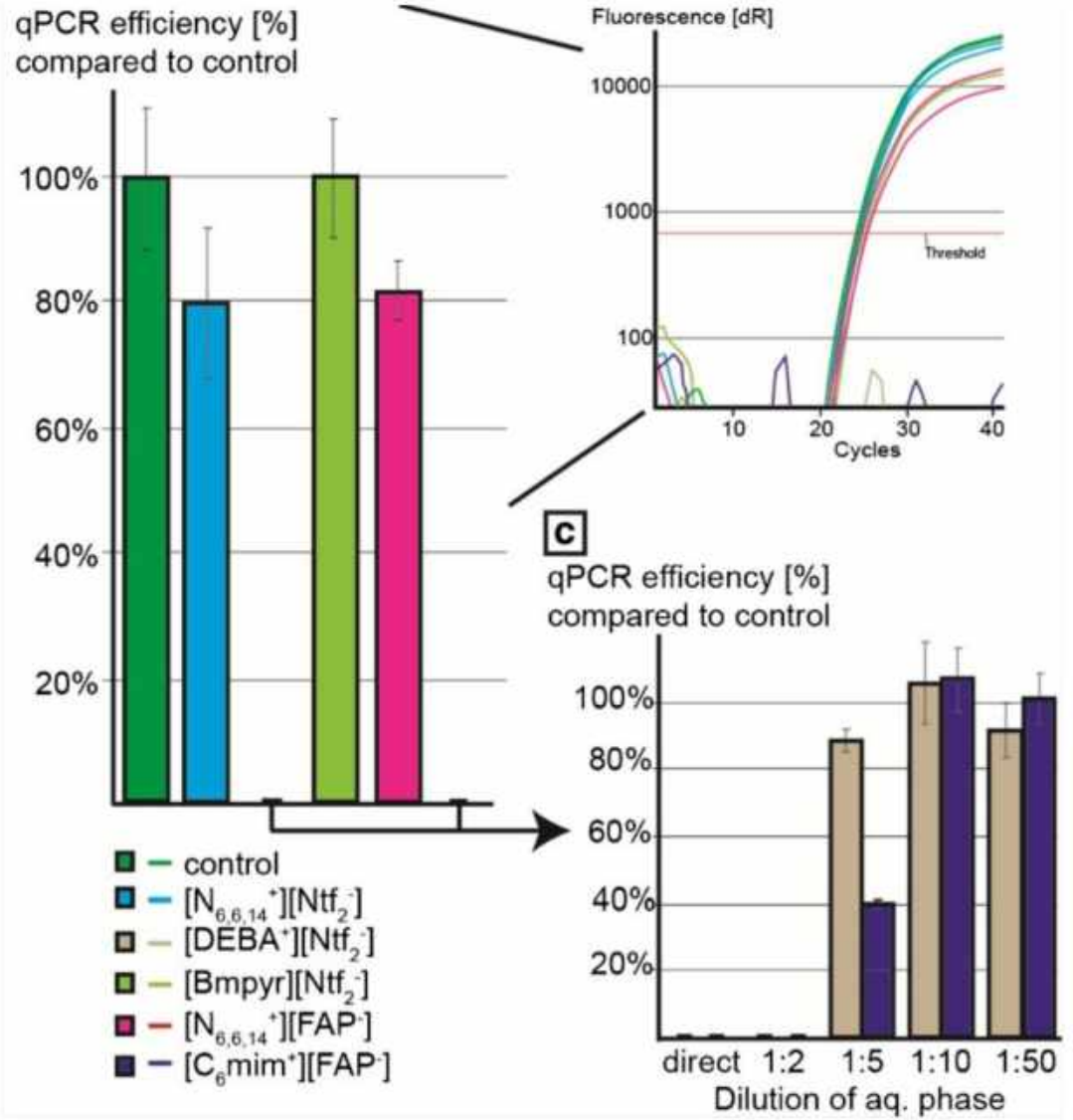
proionic

# Extraction of biomolecules by IL




- We established several liquid-liquid phase systems and used them successfully for
  - DNA extraction from bacteria, viruses and complex organic samples
  - Purification and concentration of viral particles

# Effect is IL specific




Anal Bioanal Chem (2017) 409:1503–1511
DOI 10.1007/s00216-016-0112-x

PAPER IN FOREFRONT

**Hydrophobic ionic liquids for quantitative bacterial cell lysis with subsequent DNA quantification**

Sabine Fuchs-Telka[1] · Susanne Fister[1] · Patrick-Julian Mester[1] · Martin Wagner[2] · Peter Rossmanith[1,2]

# Detection of pathogens by Mass spectroscopy

Total: 4.8 h
290 min

**Listeria species confirmation**
**PCR (hly, iap PCR)**

20 samples, negative control, positive control

| | | |
|---|---|---|
| labor acitivity | 40 min | DNA extraction<br>Chelex Resin |
| labor acitivity | 40 min | 2x preparation master-mix hy & iap<br>addition of DNA samples |
| labor acitivity<br>waiting time | 5 min<br>90 min | VWR Doppio, PCR run |
| labor acitivity<br>waiting time | 25 min<br>50 min | Gel electrophoresis<br>preparation gel 25 min, addition of samples 20 min,<br>run 2x (hly, iap) 30 min |
| labor acitivity | 40 min | Recording and interpretation of data |

Total: 1.8 h
110 min

**Listeria species confirmation**
**Maldi Biotyping**

20 samples, BCG, positive control EGDe

| | | |
|---|---|---|
| labor activity | 30 min | suspension of isolates on Maldi test plate<br>application of formic acid |
| labor activity<br>waiting time | 10 min<br>10 min | addition of Maldi Matrix, air drying |
| labor activity | 30 min | sample list, measurement in Maldi Biotyper |
| labor activity | 30 min | interpretation of Maldi Data, Export pdf to xls |

File

# Listeria monocytogenes Genotyping and Transmission Mapping Applying 3-Allel Typing

DNA extraction
Chelex Resin

PCR Mastermix
abcZ, bglA, ldh

Interpretation of Halffraser & Fraser results
(Rapid I mono Agar)

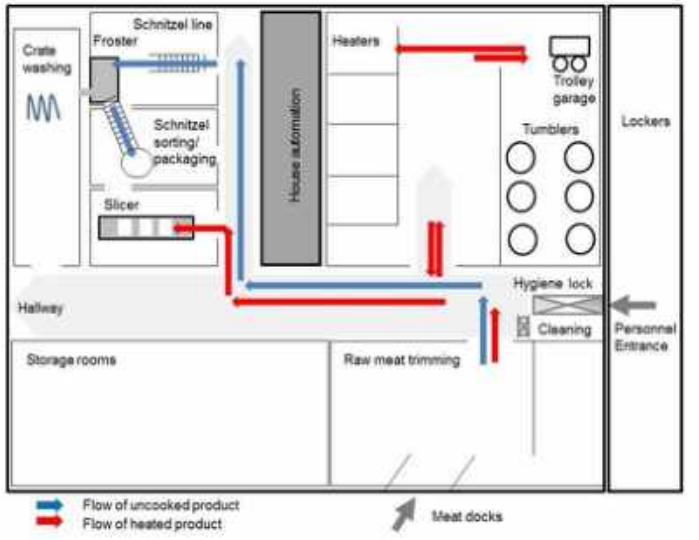
Subcultivation L.monocytogenes: up to 5 colonies

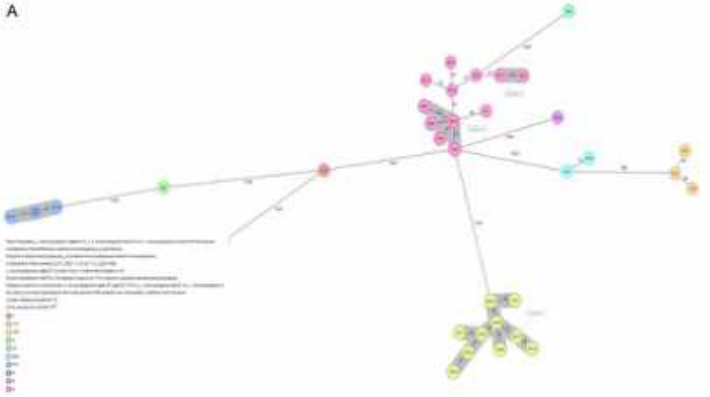
Sanger Sequencing

Interpretation of results
Prediction of Clonal Complex

Transmission mapping of CCs

Categorization of sporadic, recurrent, or persistent genotypes




Created inhttps://BioRender.com

Stessl, B., Ruppitsch, W., & Wagner, M. (2022). Listeria monocytogenes post-outbreak management—When could a food production be considered under control again? *International Journal of Food Microbiology*, *379*, 109844.

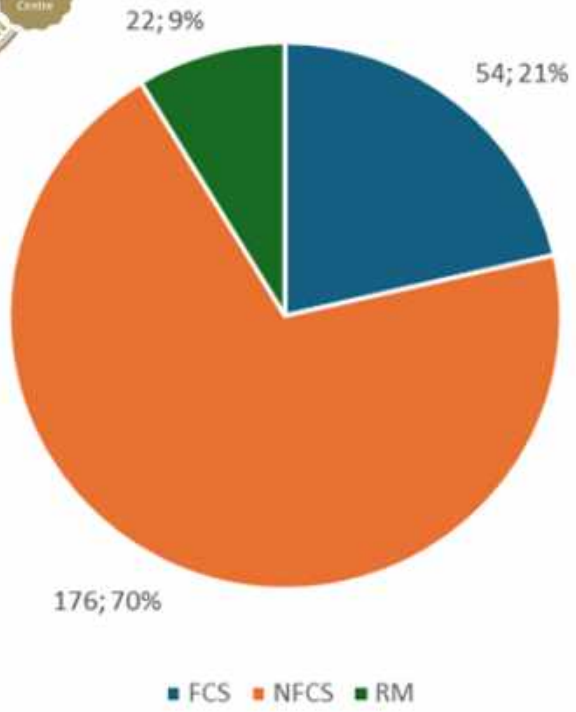
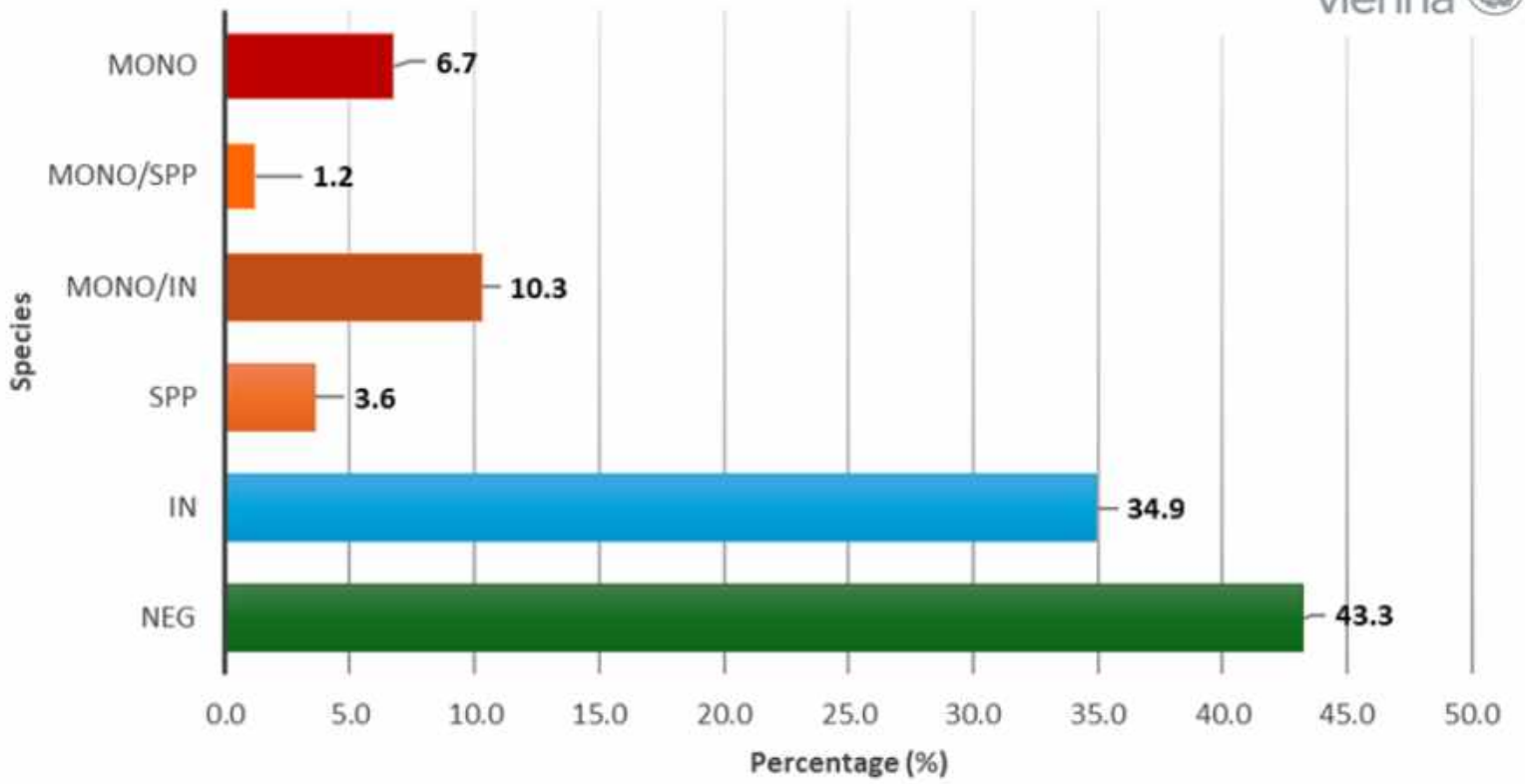



**Figure. Distribution of sample categories within the dataset (n=252).**
NFCS = Non-food contact surfaces; FCS = Food contact surfaces; RM = Raw material.

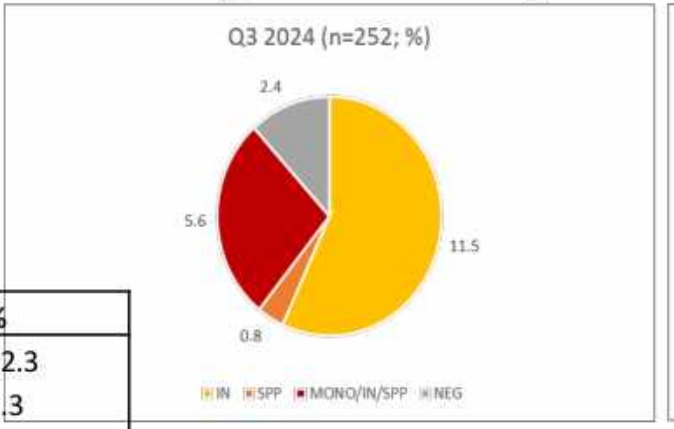


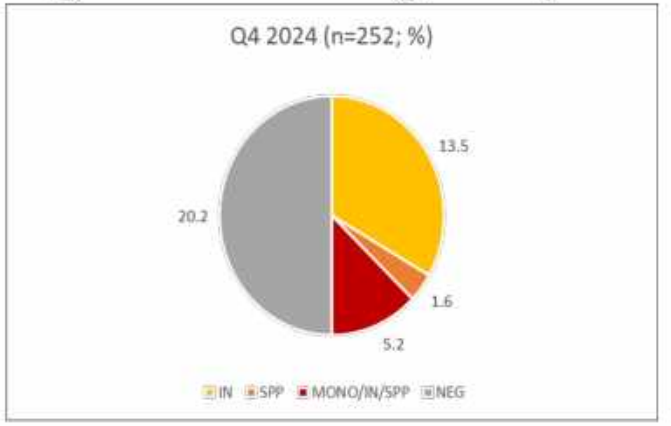

**Species identification according to ISO 11290-1 and MALDI biotyping of *Listeria* isolates in 252 samples.**
MONO, *Listeria monocytogenes*; IN, *Listeria innocua*; SPP, *Listeria* spp.; NEG, negative.

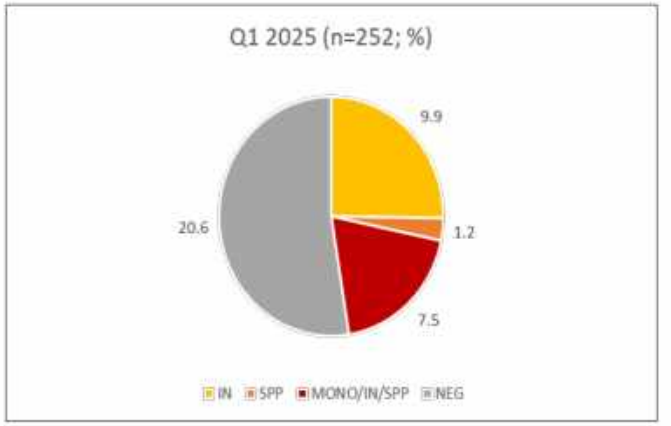
# Clonal complexes (CC) and their genetically assigned lineages (n=44).

| Lineage | CC | *abcZ* | *bglA* | *ldh* | n | % |
|---|---|---|---|---|---|---|
| I | 6 | 3 | 9 | 1 | 23 | 52.3 |
| I | 1 | 3 | 1 | 1 | 1 | 2.3 |
| I | 224 | 11 | 3 | 94 | 1 | 2.3 |
| II | 121 | 7 | 6 | 37 | 14 | 31.8 |
| II | 8 | 5 | 6 | 3 | 3 | 6.8 |
| II | 9 | 6 | 5 | 4 | 1 | 2.3 |
| II | 375 | 7 | 6 | 96 | | |
| | | | | | 1 | 2.3 |

CC, clonal complex; *abcZ*, ATP-binding cassette transporter gene; *bglA*, beta-glucosidase gene; *ldh*, lactate dehydrogenase gene.

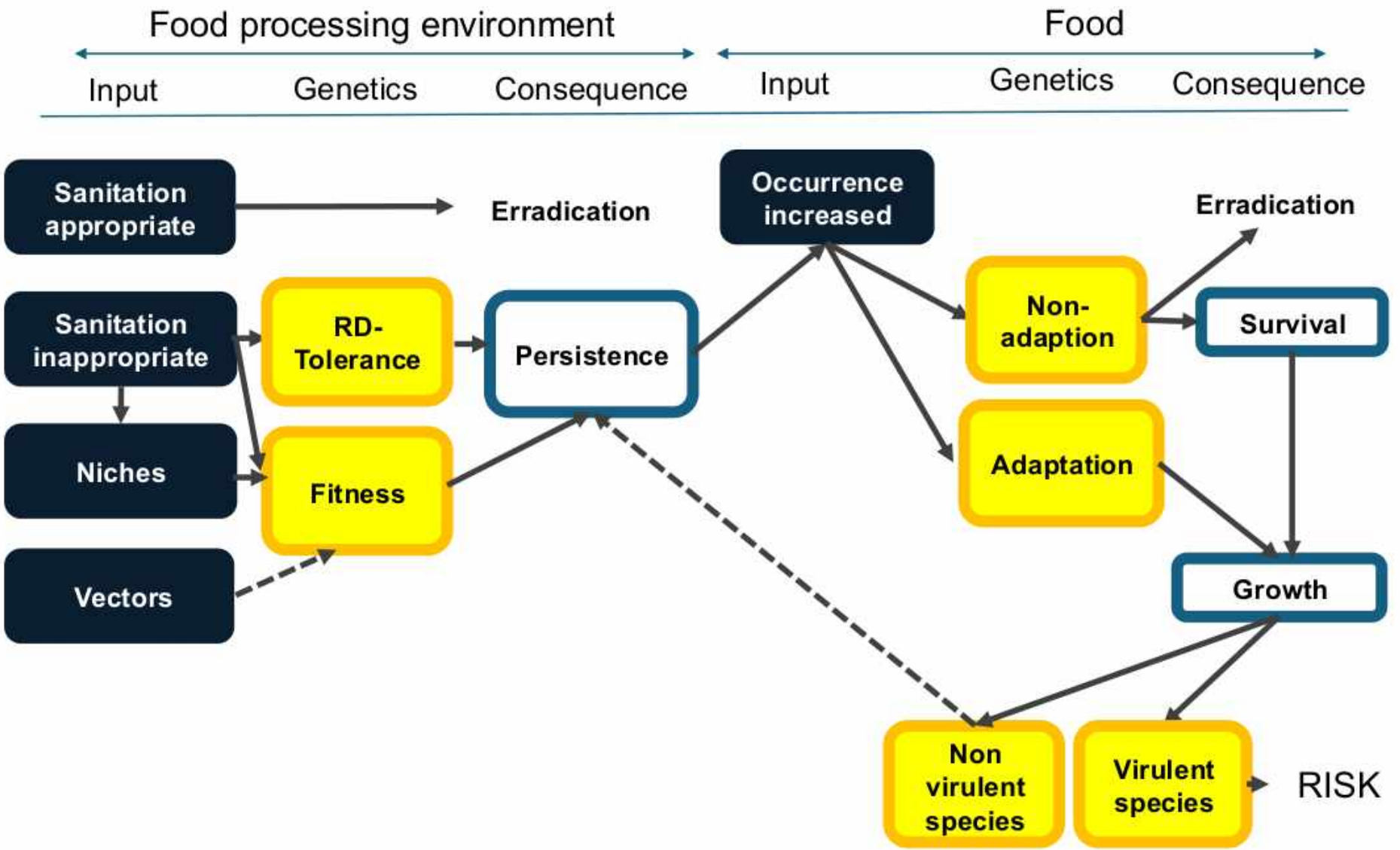



Comparison of the distribution of results from 252 samples across different time periods: third and fourth quarters of 2024 and first quarter of 2025. MONO, *Listeria monocytogenes*; IN, *Listeria innocua*; SPP, *Listeria* spp; NEG, negative.

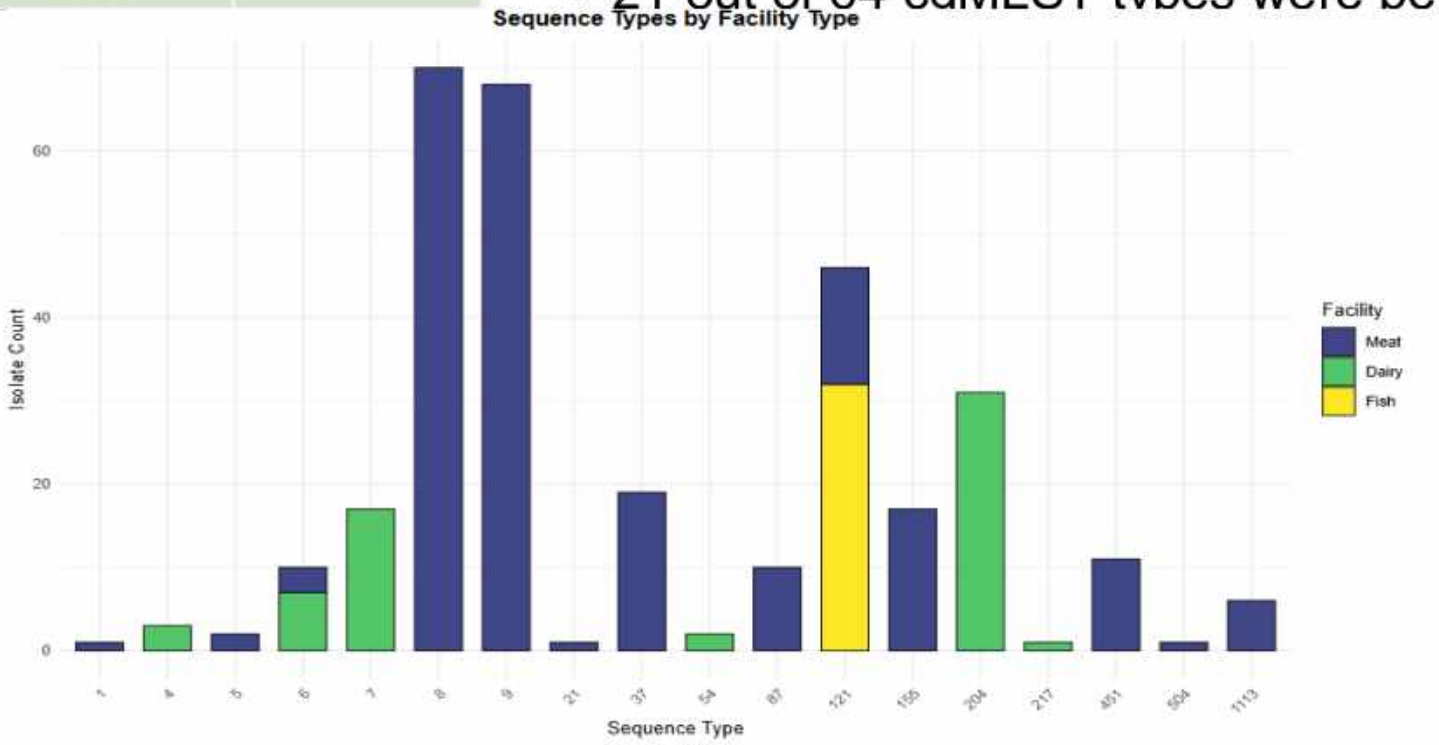
# Why Listeria as a model organism…?



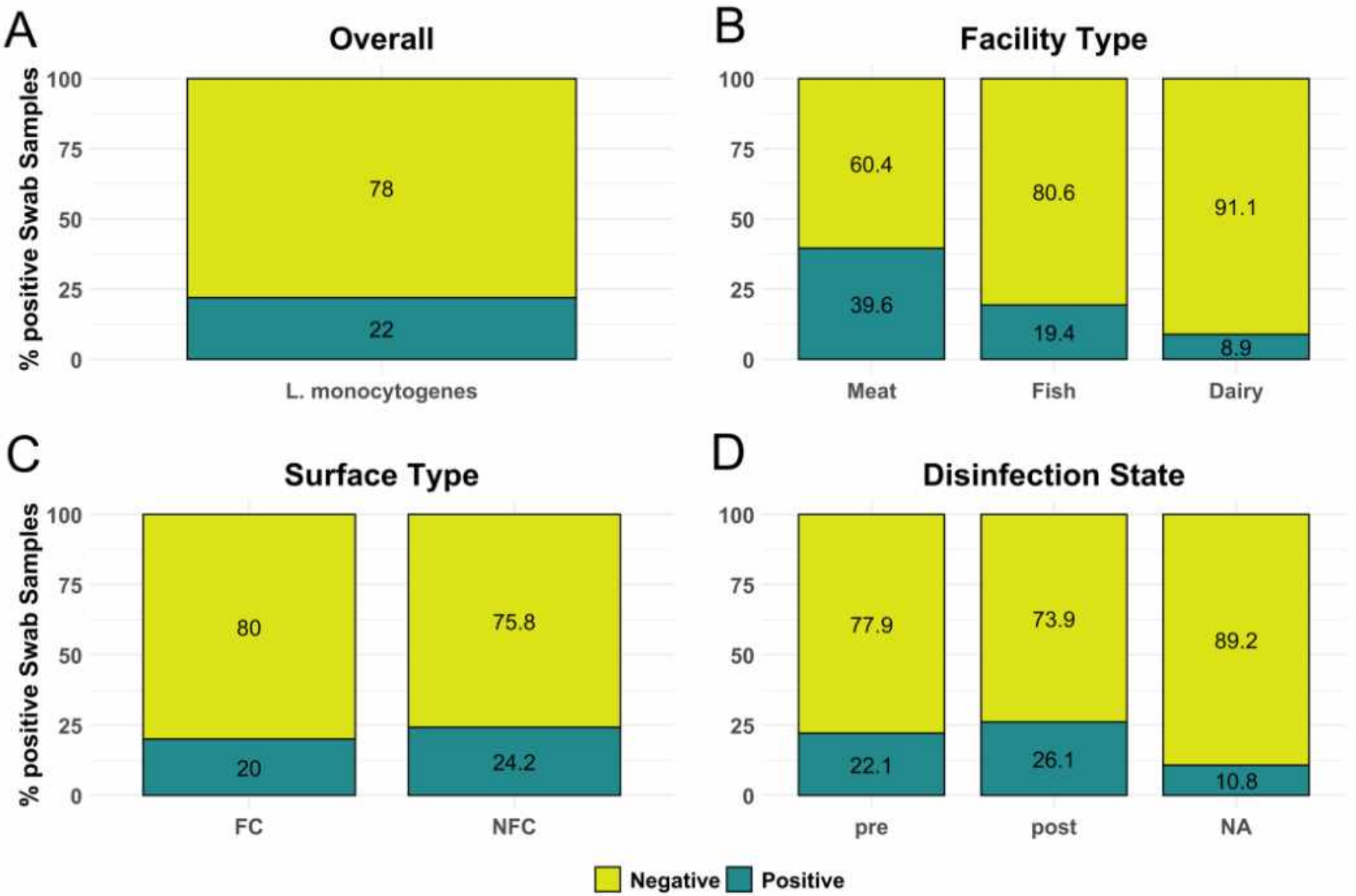
# Persistence in European FBOs

| Partner | Country | Facility Type | Facility Code |
|---|---|---|---|
| AUA | Greece | 2 Meat | A, B |
| FFoQSI | Austria | 1 Dairy, 1 Fish | C, D |
| UBU | Spain | 5 Dairy, 2 Meat | E -K |

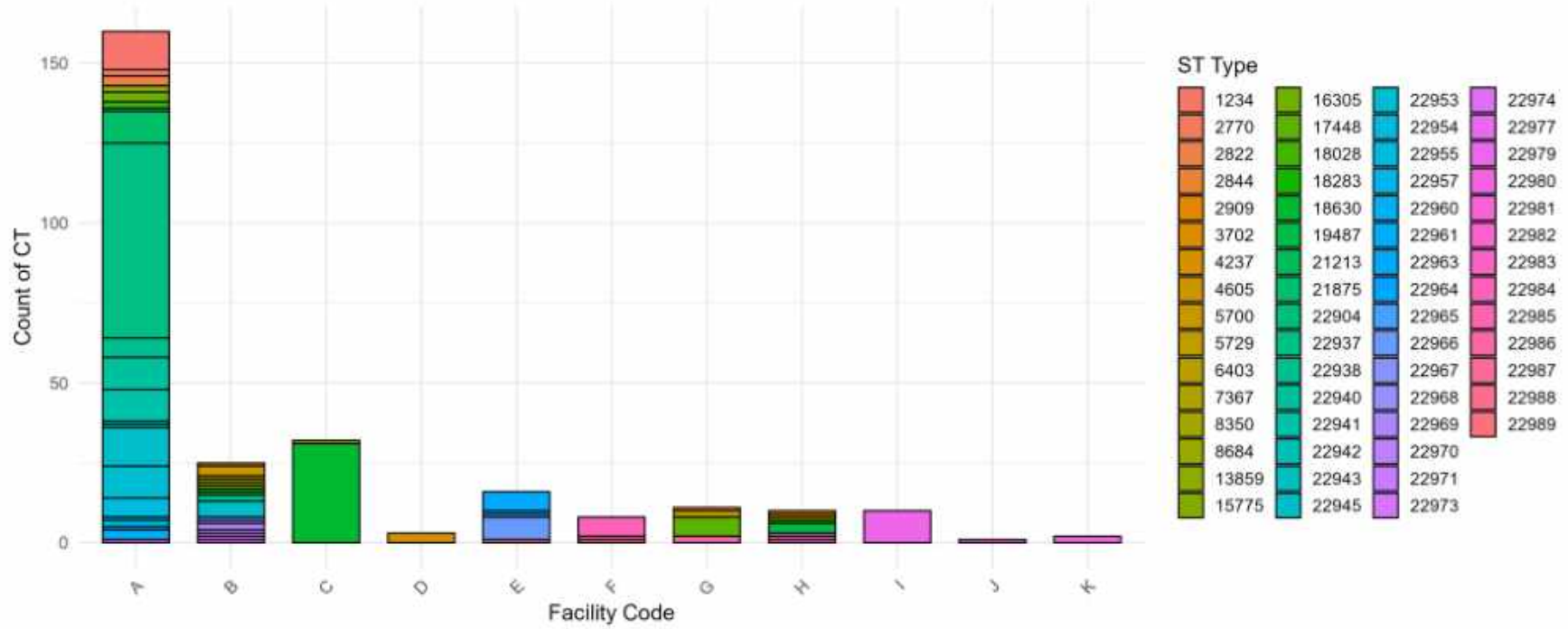
- Overall, 11 facilities sampled
- 4 Meat facilities, 6 Dairy facilities and 1 Fish facility
- In total 1194 swab samples taken
- 21% swabs positive for *L. monocytogenes*
- 21 out of 64 cgMLST types were persisters



# Occurrence in dependence of production factors

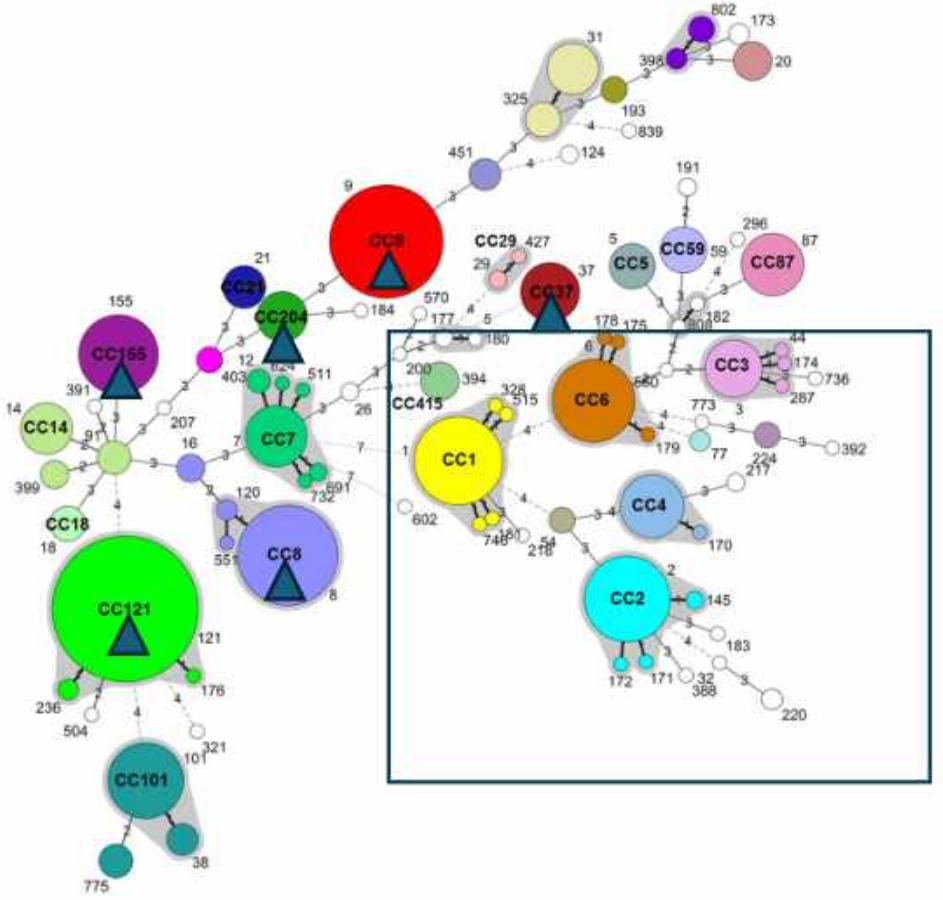


# Persistence is a multispecies phenomenon




21 out of 64 CC found: persistent

# The Persistence phenomenon




Nielsen et et al.,

Summary of Persistent Clusters

| Facility Code | Facility Type | Cluster | Lineage | ST | Clonal Complex | CT | Count |
|---|---|---|---|---|---|---|---|
| A | Meat | 1 | 1 | 155 | 155 | 1234 | 6 |
| A | Meat | 2 | 2 | 1113 | 9 | 22938 | 5 |
| A | Meat | 1 | 2 | 451 | 11 | 21875 | 10 |
| A | Meat | 1 | 2 | 8 | 8 | 22937 | 60 |
| A | Meat | 1 | 2 | 8 | 8 | 22954 | 6 |
| A | Meat | 1 | 2 | 9 | 9 | 16305 | 3 |
| A | Meat | 2 | 2 | 9 | 9 | 22940 | 5 |
| A | Meat | 4 | 2 | 9 | 9 | 22940 | 3 |
| A | Meat | 1 | 2 | 9 | 9 | 22945 | 12 |
| A | Meat | 1 | 2 | 9 | 9 | 22953 | 10 |
| C | Fish | 1 | 2 | 121 | 121 | 18630 | 31 |
| D | Dairy | 1 | 1 | 4 | 4 | 4605 | 3 |
| E | Dairy | 3 | 2 | 204 | 204 | 22966 | 4 |
| E | Dairy | 4 | 2 | 204 | 204 | 22966 | 3 |
| G | Meat | 1 | 2 | 37 | 37 | 17448 | 7 |
| H | Meat | 1 | 1 | 87 | 87 | 19487 | 6 |
| H | Meat | 1 | 2 | 121 | 121 | 8684 | 3 |

# Microbial anaytical platforms : Key messages

- Still nowadays, culture-based methods are gold standards for public health labs
- Emerging microbial hazards need to be tackeled by a method tool box (collecting quantitative, typing and genetic information (quasimetagenomics)
- Industry needs rapid technology (nevertheless enrichments remains the key)
- Of importance is to see the integrity of the analytical chains (Sampling, sample treatment, analyte isolation, analyte confirmation)
- Price is the main driver but easy of manipulation is a limiting factor