International Association for Food Protection® WEBINAR

Is it a Listeria sensu stricto or sensu lato species? Why understanding the difference is important

Organized by: The Applied Laboratory Methods PDG and Merieux NutriSciences

Moderator: **Sarita Raengpradub,** Merieux NutriSciences

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International Association for Food Protection WEBINAR

Today's Moderator:

Sarita Raengpradub, Ph.D.

Dr. Sarita Raengpradub Wheeler is Director of Microbiology R&D with Mérieux NutriSciences. She received her B.S. in Biology from the University of Illinois and her Ph.D. in Food Science from Cornell University with emphasis on molecular microbiology and food safety. She now directs the Corporate Microbiology R&D department for Mérieux NutriSciences North America and supports Global Biology Innovation activities as a part of the Analytical Hub.

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Martin Wiedmann

Martin Wiedmann, Dr. med. vet, Ph.D.
Gellert Family Professor of Food Safety
Cornell University

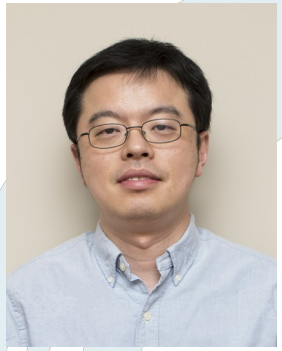
Martin received a veterinary degree and a doctorate in Veterinary Medicine from the Ludwig-Maximilians University in Munich, and a Ph.D. in Food Science from Cornell, where he currently is the *Gellert Family Professor of Food Safety*. His teams research interests focus on farm-to-table microbial food quality and food safety and the application of molecular and modelling tools to study the transmission of foodborne pathogens and spoilage organisms, including translation of the associated research findings into reducing foodborne illnesses and food spoilage. Students and staff that were previously associated with his team have pursued successful careers in a range of environments, including industry, government, academia, and non-for profits.

Catharine Carlin

Catharine Carlin, Ph.D.
Director of Microbiology Innovation
Mérieux NutriSciences

Catharine received a B.S. in Biology from the Ohio State University (2001), and a Ph.D. in Food Science from Cornell University (2021). In total, Catharine has worked in the food industry for over 20 years, all with Mérieux NutriSciences (formerly Silliker Labs). Throughout her time with Mérieux NutriSciences, Catharine has held several different positions within the microbiology food testing and research departments. Her recent graduate work with the Wiedmann Food Safety Laboratory included the characterization of six novel *Listeria* spp. Her primary research area, including current projects, is focused on foodborne pathogen detection and identification methods. Catharine is an active contributor of microbiology method development with AOAC, several ISO TC 34/ SC 9 working groups, and MicroVal.

Yi Chen



Yi Chen
Food and Drug Administration (FDA)

Dr. Yi Chen is currently a research microbiologist and has been with the Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) Division of Microbiology since 2008. He is the FDA subject matter expert for *Listeria monocytogenes* and *Cronobacter*. Dr. Chen led development and validation efforts on the detection, enumeration and whole genome sequencing analysis of these two pathogens. He has also represented FDA and served as collaborators on ISO method validation efforts. Dr. Chen is the co-author of the *Listeria* and *Cronobacter* chapters of FDA *Bacteriological Analytical Manual*. As the subject matter expert, he has provided scientific advice for various FDA assignments, outbreak investigations and laboratory analyses. Dr. Chen received his Ph.D. in Food Science from the Department of Food Science at the Pennsylvania State University in 2007. He currently serves as a member of Microbial Method Validation Subcommittee of FDA, Expert Review Panel for AOAC International, ISO *L. monocytogenes* working group, and Editorial Board member for Applied and Environmental Microbiology.

“New“ *Listeria* species - Are they even *Listeria*?

Genomics data suggests only the *Listeria* sensu stricto species should be classified in the genus

Martin Wiedmann
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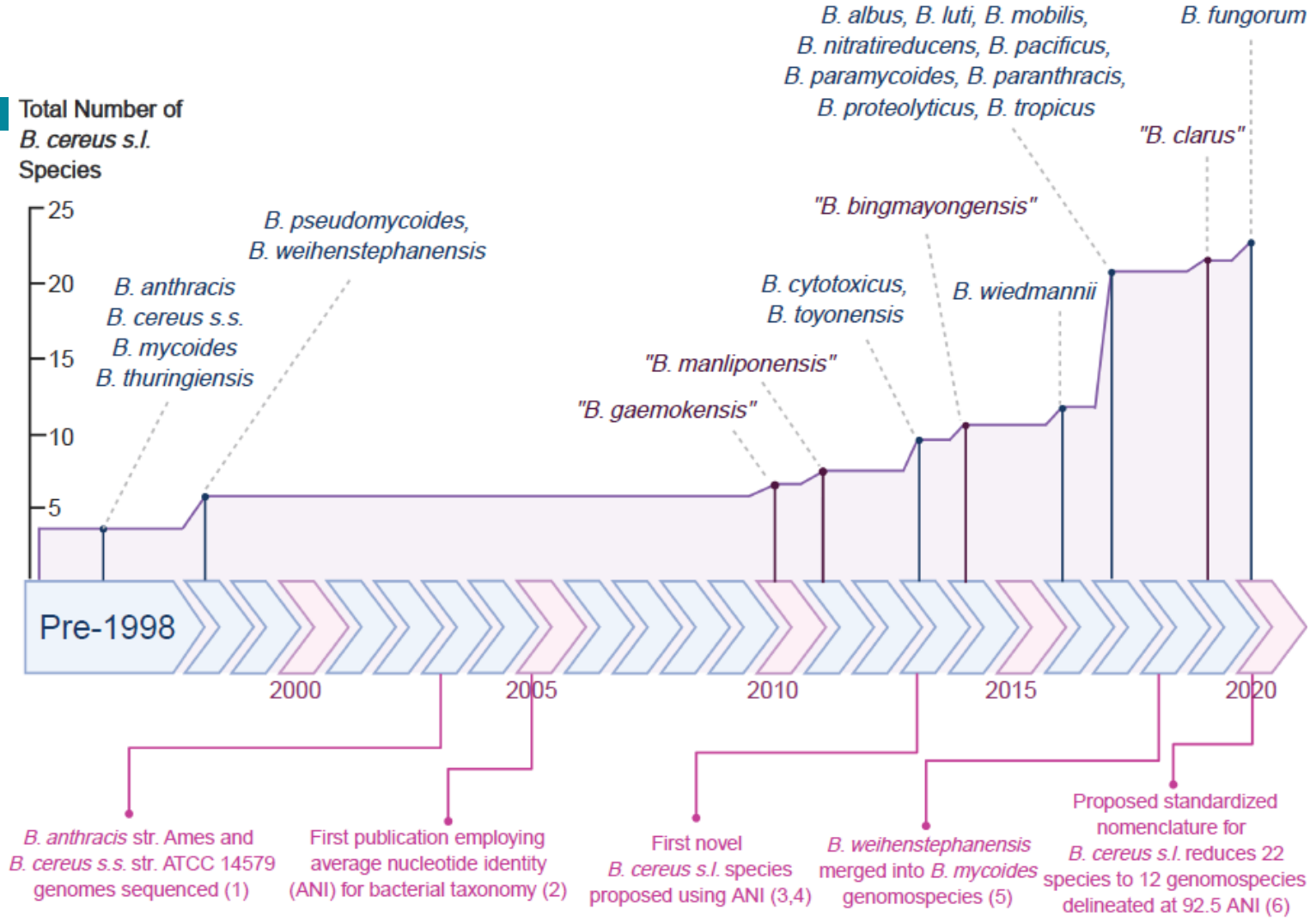
Outline

- **The bigger picture: Impact of WGS on bacterial taxonomy**
- New *Listeria* species – where are we now
- Where do we go from here
- How do we deal with this “right now”

The bigger picture

- Species definition often used to define what is and isn't a microbial hazard
- WGS has changed many things, including how we define species
  - New species, new genera, renaming species and genera are the "new normal"
    - Food safety and public health may not always be able to keep up with these changes
  - Classifying a taxonomic group as "hazard" is not as easy as it was
    - "*L. monocytogenes*" versus "*L. monocytogenes plus hemolytic L. innocua*" versus "*L. monocytogenes plus hemolytic L. innocua plus L. ivanovii*"
  - Need to rethink how we define bacterial groups that are food safety relevant "test targets" (e.g., *Listeria*)

Twenty years of *B. cereus*



L. M. Carroll, R. A. Cheng, M. Wiedmann & J. Kovac (2021): Keeping up with the *Bacillus cereus* group: taxonomy through the genomics era and beyond, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1916735

Outline

- The bigger picture: Impact of WGS on bacterial taxonomy
- **New *Listeria* species – where are we now**
- Where do we go from here
- How do we deal with this “right now”

Listeria Intro

- *Listeria monocytogenes* typically considered the only human pathogen
- However, samples collected from processing plant environments and other food associated environments (e.g., packing houses) are often tested for *Listeria* spp. to identify conditions where *Lm* could be introduced or survive (“persist”)

Listeria species - where are we today

- 1926 – 2009: 6 species
  - *L. monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, L. grayi*
  - ***L. grayi*** – divergent from LM compared to the other species, placement in the genus debated
    - "This tree clearly shows that the newly characterized strain CIP 109804 [L. rocourtiae] … belongs to the genus *Listeria* as long as this genus includes *L. grayi* "
- 2009 – 2023: 22 species added for a total of 28 species
  - Latest additions are *Listeria ilorinensis* and *Listeria swaminanthanii*
  - The genus *Listeria* has been grouped into ***Listeria sensu stricto*** (species closely related to LM) and ***Listeria sensu lato*** (distantly related to LM)
    - Some (including yours truly) argue that *Listeria sensu lato* should probably should not be even considered to be part of the genus *Listeria*

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Raufu *et al.*, *Int. J. Syst. Evol. Microbiol.* 2022;72:005437
DOI 10.1099/ijsem.0.005437

MICROBIOLOGY SOCIETY

Listeria ilorinensis sp. nov., isolated from cow milk cheese in Nigeria

Ibrahim Adisa Raufu[1]†, Alexandra Moura[2,3]†, Guillaume Vales[2,3], Olayiwola Akeem Ahmed[1], Abdulfatai Aremu[4], Pierre Thouvenot[2,3], Nathalie Tessaud-Rita[2,3], Hélène Bracq-Dieye[2,3], Ramar Krishnamurthy[5], Alexandre Leclercq[2,3] and Marc Lecuit[2,3,6,*]

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE

Soil Collected in the Great Smoky Mountains National Park Yielded a Novel *Listeria sensu stricto* Species, *L. swaminathanii*

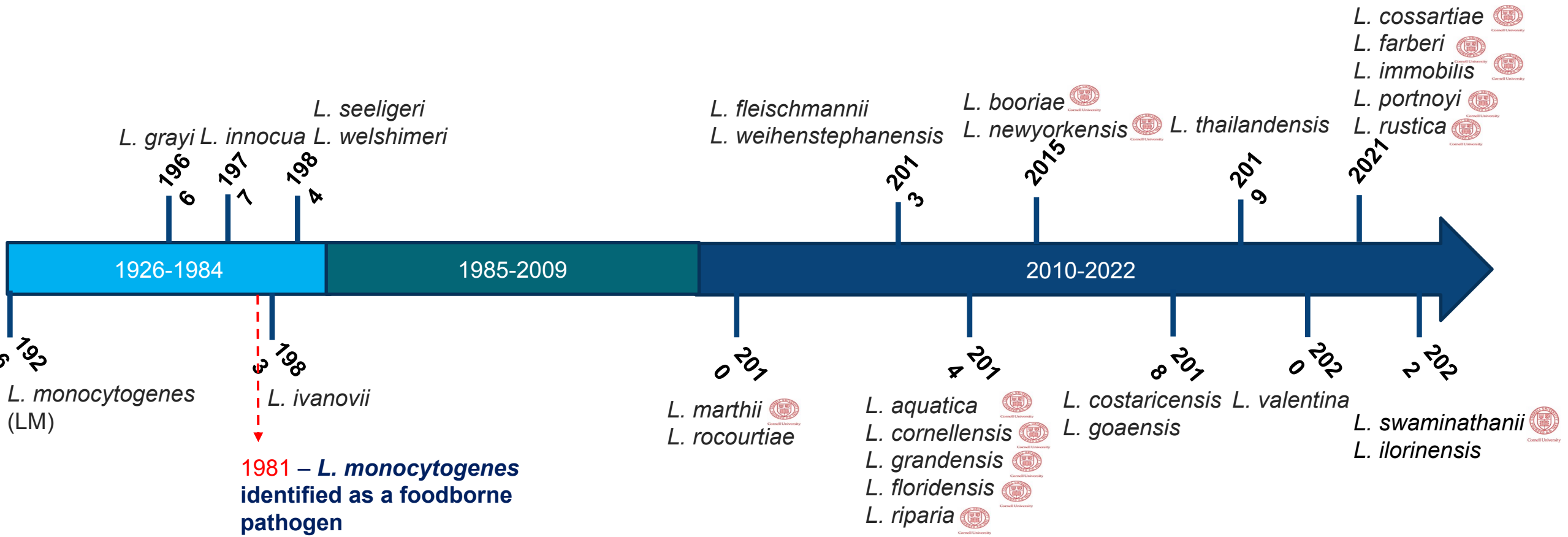
Catharine R. Carlin,[a] Jingqiu Liao,[a,b*] Lauren K. Hudson,[c] Tracey L. Peters,[c] Thomas G. Denes,[c] Renato H. Orsi,[a] Xiaodong Guo,[a] Martin Wiedmann[a]

CornellCALS College of Agriculture and Life Sciences

Listeria – from 6 to 28

Cornell University

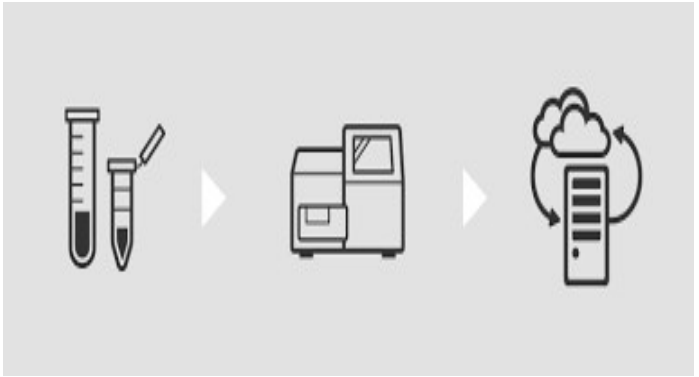
Logo indicates species was identified by the Cornell University Wiedmann lab



1981 – ***L. monocytogenes*** **identified as a foodborne pathogen**

Why so many “new” species?

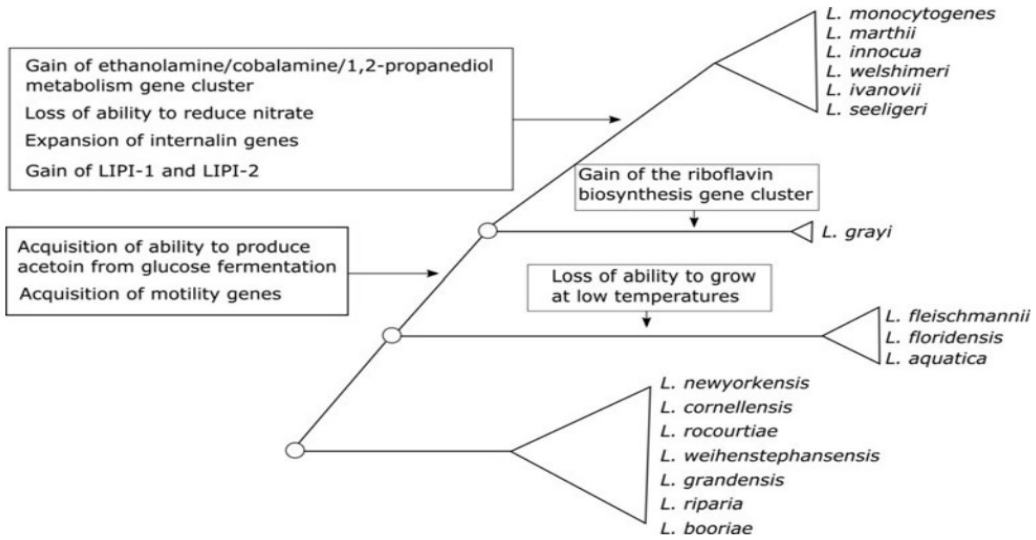
- *sigB* sequencing-based ID – a reliable subtyping tool
- Advances in Whole-genome sequencing (WGS)
  - Faster and cheaper
- Development of rapid WGS species classification tools
  - Automated computational tools
- Identification of novel species, novel genera, novel subspecies, re-classification
  - Expect this to continue



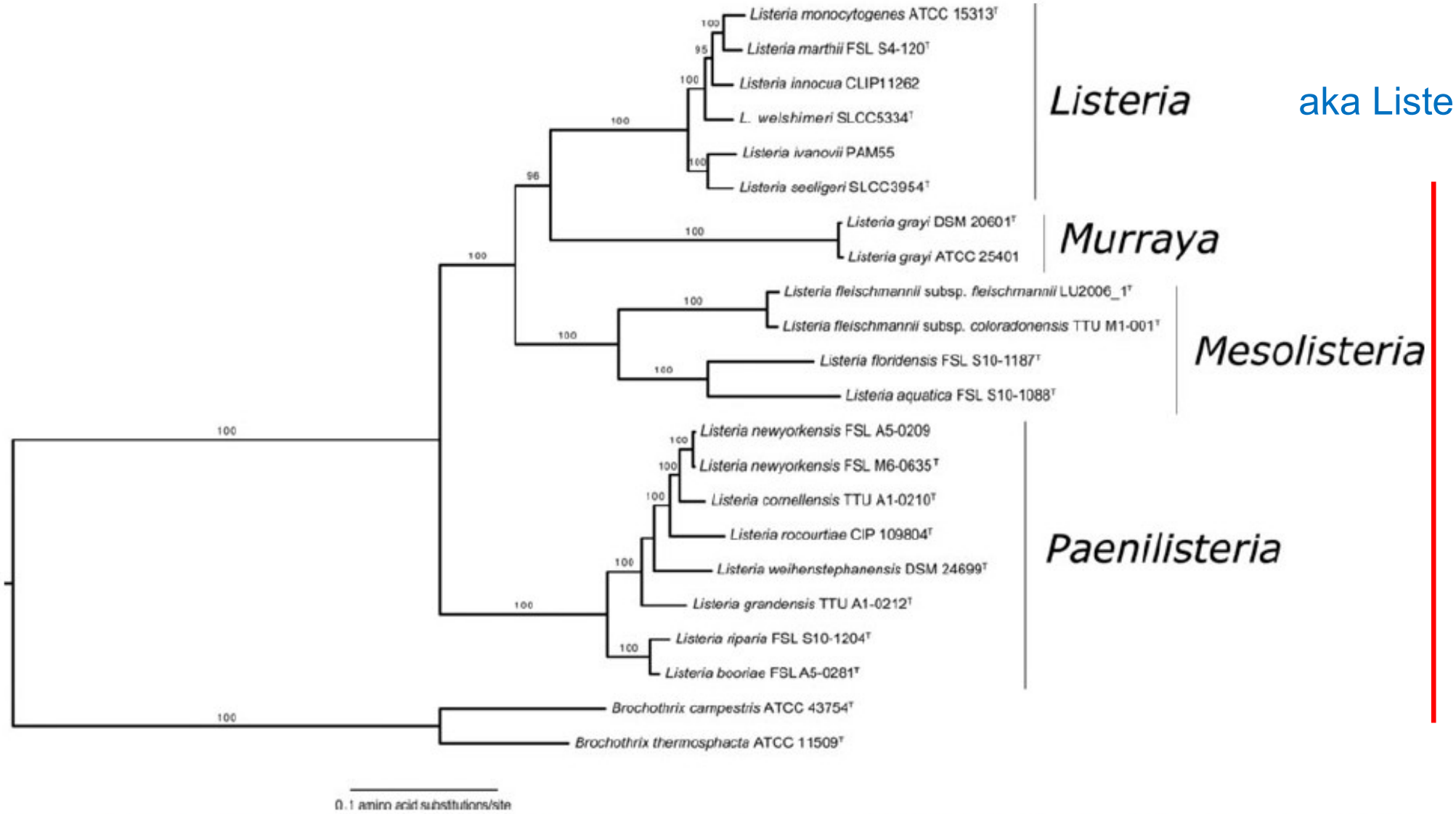
Isolate → Sequence → Analyze

Listeria – Summary through 2020

- By 2020 there were 21 *Listeria* species
- 14 out the 15 recently added species grouped into the *sensu lato* clade
- No additional pathogenic species
- *Sensu lato* characteristics are divergent enough they are arguably not *Listeria*



Orsi. R. and Wiedmann, M (2016)



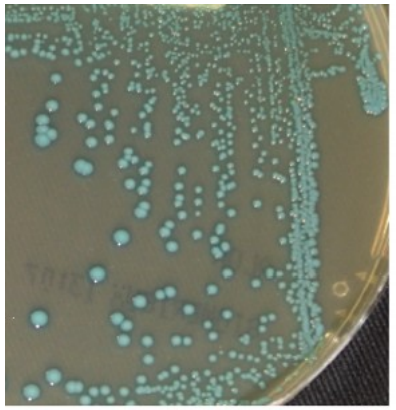
aka Listeria *sensu stricto*

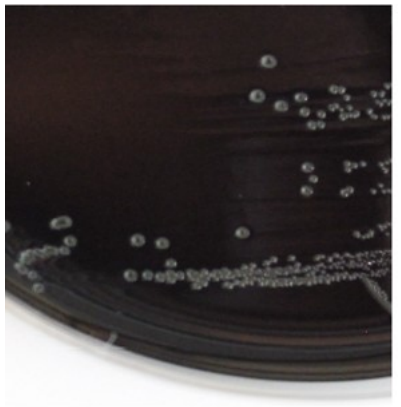
aka Listeria *sensu lato*

Fig. 1 Phylogenetic tree modified from Weller et al. (2015). Maximum represent bootstrap values (>70 %) based on 250 bootstrap replicates.

Novel Species - Summary

- 7 new *Listeria* species reported in 2021 and 2022
  - 4 *sensu stricto* species
- 22 new *Listeria* species since 2009
  - No additional pathogenic species, but 5 of these new species are *Listeria sensu stricto*
  - Current confirmation methods may not detect or misidentify a number of them
    - Lack of motility as one issue
    - Novel biochemical profile (catalase, motility, sugar fermentation, etc.)
  - How do these species grow relative others → largely unknown
  - Some may outcompete Lm in enrichment media
    - Some species undetectable by rapid methods may outgrow species detected by rapid methods -> risk of false negatives with *Listeria* spp. assays
- Ignoring these new species simply because "they are rare in food and processing facilities" may neither be scientifically correct nor prudent





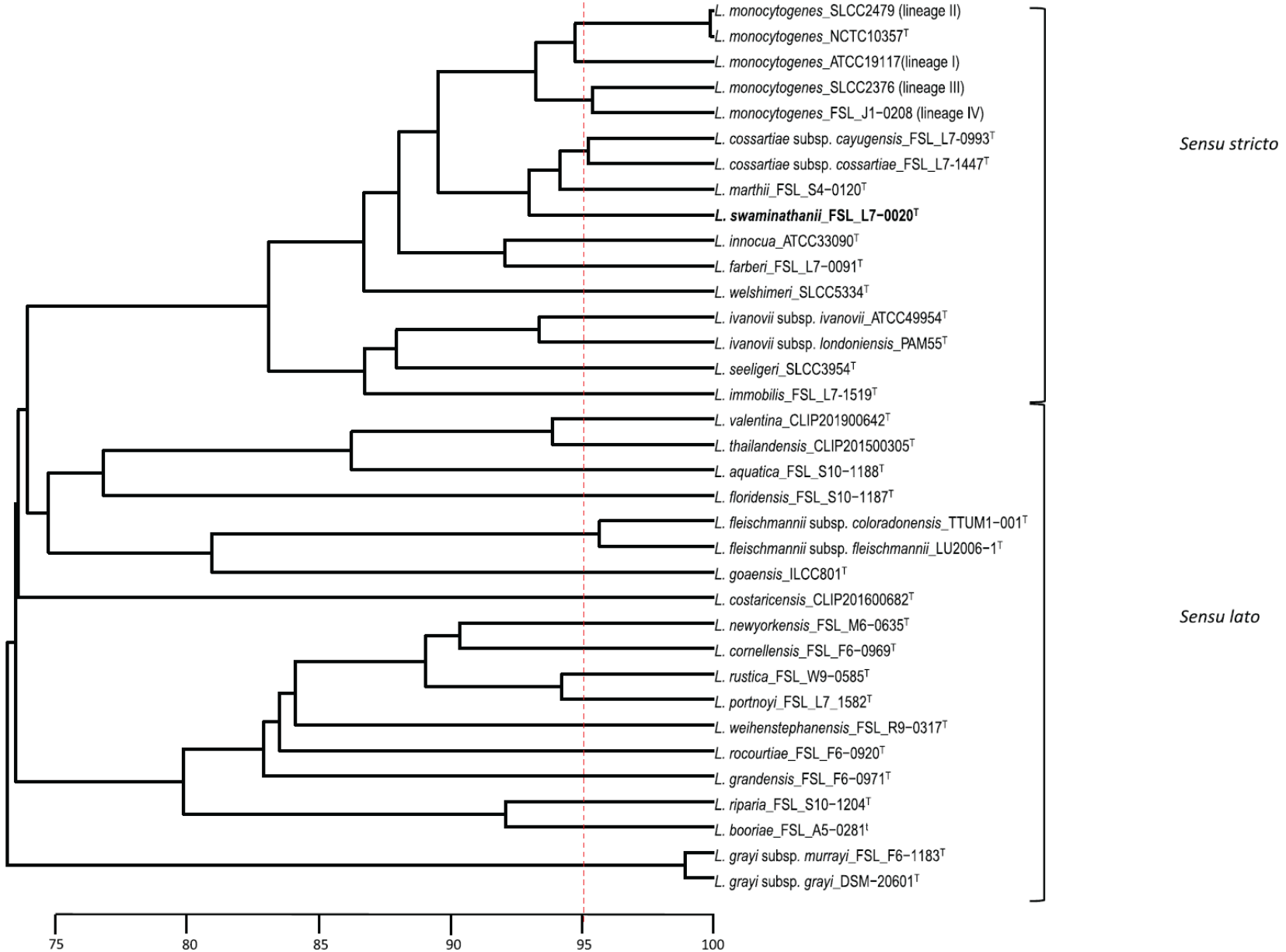


FIG 1 UPGMA dendrogram based on Average Nucleotide Identity BLAST (ANIb) analysis of 34 reference genomes (consisting of the 30 *Listeria* species and subspecies type strains described as of June 11, 2021, and one genome representing each of the four *L. monocytogenes* lineages) and the *L. swaminathanii* FSL L7-0020^T draft genome. The vertical red dotted line is placed at 95%, representing the species cutoff. The horizontal scale bar indicates ANI percentage similarity.

Are all 27 non-LM *Listeria spp.* index organisms?

- **Likely ONLY the *sensu stricto* species**
  - *L. innocua*
  - *L. ivanovii*
  - *L. seeligeri*
  - *L. welshimeri*
  - *L. marthii*
  - *L. cossartiae*
  - *L. immobilis*
  - *L. farberi*
  - *L. swaminathanii*

- **The *sensu lato* likely NOT index organisms**
  - *L. aquatica*
  - *L. booriae*
  - *L. cornellensis*
  - *L. costaricensis*
  - *L. floridensis*
  - *L. fleischmannii*
  - *L. grandensis*
  - *L. grayi*
  - *L. goaensis*
  - *L. newyorkensis*
  - *L. portnoyi*
  - *L. rocourtiae*
  - *L. riparia*
  - *L. rustica*
  - *L. thailandensis*
  - *L. valentina*
  - *L. weihenstephanensis*
  - *Listeria ilorinensis*

Key take home messages (so far)

- Not all “new” *Listeria* species are distinct from *L. monocytogenes*
  - For example, *L. cossartiae* and *L. marthii* are closely related to *L. monocytogenes*
- The key differentiation should not be **“old”** and **“new“** Listeria, but ***Listeria sensu stricto*** (species closely related to LM) and ***Listeria sensu lato*** (distantly related to LM)
  - *L. grayii* is on “old” Listeria species, but represents *Listeria* sensu lato
    - If *L. grayii* reclassification as *Murraya* would have been retained, things would be lot different today

INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1987, p. 298–300
0020-7713/87/030298-03$02.00/0

Vol. 37, No. 3

Proposal to Retain *Listeria murrayi* and *Listeria grayi* in the Genus *Listeria*

JOCELYNE ROCOURT,[1] UTA WEHMEYER,[2] PASCALE COSSART,[3] AND ERKO STACKEBRANDT[2]*

Unité d'Ecologie Bactérienne[1] and Unité de Génie Microbiologique,[3] Institut Pasteur, F-75724 Paris, Cedex 15, France, and Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität, D-2300 Kiel, Federal Republic of Germany[2]

The 16S ribosomal ribonucleic acid oligonucleotide catalog of *Listeria murrayi* was found to be closely related (similarity coefficient, 0.73) to that of *L. monocytogenes*. These data, together with the phenotypic similarity found among all *Listeria* strains tested, provide no support for the exclusion of *L. murrayi* (and the closely related species *L. grayi*) from the genus *Listeria*.

Listeria grayi and *L. murrayi* were first described by Larsen and Seeliger in 1966 and Welshimer and Meredith in 1971, respectively (22; H. E. Larsen and H. P. R. Seeliger, Proc. 3rd Int. Symp. Listeriosis). According to *Bergey's Manual of Systematic Bacteriology*, *vol. 2* (15), *L. grayi* and *L. murrayi* are considered as species incertae sedis, reflecting the long-controversial view about the taxonomic position of these two species. This originated from low deoxyribonucleic acid (DNA) homology values observed by Stuart and Welshimer between *L. grayi* and *L. murrayi* on the one hand and *L. monocytogenes* on the other (20). These results were later confirmed (average of relative binding ratios ranging from 1 to 16%) (12). On the basis of percentage of DNA homology and phenotypic similarity, Stuart and Welshimer proposed to transfer *L. grayi* and *L. murrayi* to a new genus, "*Murraya*" (21), with "*M. grayi*" as the type species, divided into two subspecies, "*M. grayi* subsp. *grayi*" and "*M. grayi* subsp. *murrayi*." The two taxa shared an average DNA homology value of 68% (20). These names were not included in the Approved Lists of Bacterial Names (16) and have not been validated by publication or announcement in the *International Journal of Systematic Bacteriology*.

What do the authorities say about all of this??

- In the US and Canada, regulatory methods currently only mention the “original six”

FDA: " In recent years, many new species were proposed. However, these new species are not widely adopted and the number of type strains for the newly proposed species are very limited

- ISO added the species described as of 2016 (+11)

Outline

- The bigger picture: Impact of WGS on bacterial taxonomy
- New Listeria species – where are we now
- **Where do we go from here**
- How do we deal with this “right now”

Where do we go from here?

- Stick with the “original six”
- Retain *Listeria sensu stricto* and *Listeria sensu lato* under the genus *Listeria*
- Reclassify *Listeria sensu lato* into “non-*Listeria*” *genera*

Arguments for continued focus on the "original six"

- Why continue to only focus on the "original 6" species?
  - The majority of new species are distantly related to LM (i.e., *sensu lato*)
  - All the new species are considered to have low prevalence (not true)
  - Updating procedures takes years
- Why include *L. grayi*?
  - Historical species, not technically relevant (i.e., no good reason)

Arguments to retain *Listeria sensu stricto* and *Listeria sensu lato* under the genus *Listeria*

- Path of least resistance
- Many existing classical and rapid methods detect some *Listeria sensu lato* species

Reclassify *Listeria* sensu lato into "non-*Listeria*" genera

- Supported by genomics and phenotypic data
  - Several *Listeria sensu lato* species (8/17) do not grow at refrigeration temperatures
  - *Listeria sensu lato* species lack motility
  - Most *Listeria sensu lato* species (11/17) reduce nitrate
- Will remove species that are phenotypically dissimilar from *L. monocytogenes*
- May require re-design of methods that detect (some) *Listeria sensu lato* species

Aren’t there any guidelines as to what is and is not a genus?

- No current unambiguous or agreed upon way to delineate bacterial genera
- Proposed approaches include:
  - amino acid identity (AAI)
  - percentage of conserved proteins (POCP)
  - 16S rRNA gene identity: 94.5 to 95% threshold
  - Genomic coherence based on Microbial Species Identifier (MiSI), which employs both alignment fractions (AF) and average nucleotide identity (ANI)

Outline

- The bigger picture: Impact of WGS on bacterial taxonomy
- New *Listeria* species – where are we now
- Where do we go from here
- **How do we deal with this "right now"**

What to do today

- Test detection methods with all current *Listeria* species
  - There even is a possibility of false positives with *L. monocytogenes* tests and confirmation methods
  - Request data on assay specificity
- Be prepared to use WGS for confirmation of *Listeria* spp. and *L. monocytogenes* isolates
  - Requires an up to date and well annotated database
    - If your database does not include *L. swaminathanii*, WGS data analyses will not identify your isolates as *L. swaminathanii*
  - Different computational approaches can be used for species ID
  - Average nucleotide identity by BLAST (ANIb)
  - in silico DNA-DNA Hybridization
  - Genome Taxonomy Database Toolkit (GTDB-Tk)

Summary and conclusions

- The diverse range of *Listeria* species can cause challenges with detection methods, both classical and rapid
- The genus *Listeria* has been grouped into ***Listeria sensu stricto*** (species closely related to LM) and ***Listeria sensu lato*** (distantly related to LM)
  - *Listeria sensu lato* species are distinct from *L. monocytogenes;* detection of these species may have limited value for indicating conditions where *L. monocytogenes* is likely to be able to persist or be present
    - Detection methods should be validated to detect all *Listeria sensu stricto*; inability to detect sensu lato species is not a big issue
  - Hope is the *Listeria sensu lato* species are re-assigned into "Non-*Listeria*" genera
- Key industry watch outs:
  - *Listeria* spp. test "false positive" and "false negatives" – make sure you/your lab has well designed confirmation procedures, including appropriate procedures for *Listeria* speciation

Acknowledgments

- **Students and staff (current and past)**: Dr. Renato Orsi, Dr. Daniel Weller, Sherry Roof, Dr. Henk den Bakker
- **Collaborators**: Dr. Catharine Carlin

Thank You

- Questions?

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Better Food. Better Health. Better World.

Is it okay if a *Listeria* spp. rapid detection method doesn't detect all species?

Catharine Carlin, Ph.D.

Agenda

- Rapid detection methods & detection of all *Listeria* expectations
  - Inclusivity data
  - Growth in enrichment
- Cultural confirmation
  - Genus-level confirmation
  - Species identification
- Key takeaways

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First, a quick re-cap

- *Listeria sensu stricto*
  - *L. monocytogenes* and the 9 species that are similar to LM
  - The *Listeria sensu stricto* should be the target of an LM Environmental Monitoring Program (EMP)
- *Listeria sensu lato*
  - The species that are different enough from LM to warrant classification into separate genera
  - To-date, no association to a potential for LM contamination

Rapid Detection Methods

Expectations for detection of all *Listeria* spp.

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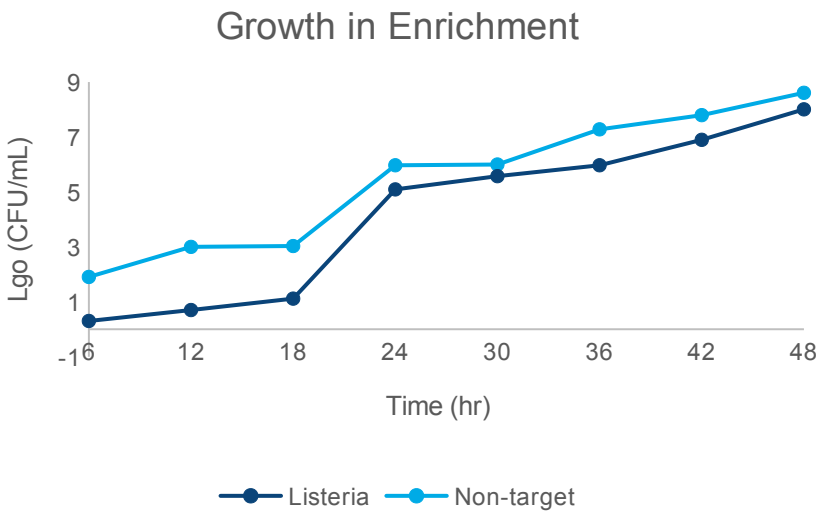
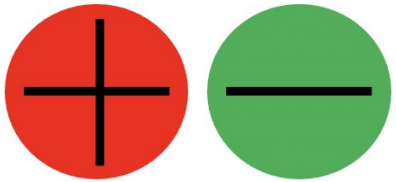
Rapid detection methods

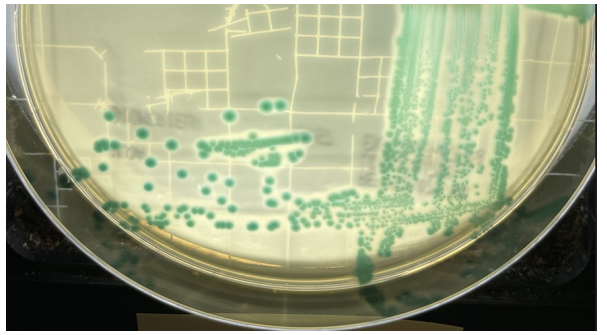
Enrichment (24-48 h)

Detection (15 min - 4 h)

Cultural Confirmation (2 – 5 d)







Rapid detection methods

- **Two main types**

1. **Molecular-based**
   - Target: a nucleic acid sequence common to all *Listeria* spp.
   - Sequences common to *Listeria sensu stricto* are typically divergent in *sensu lato*
2. **Immunoassay-based**
   - Target: typically, flagella protein (motility)
   - *Listeria sensu lato* are non-motile

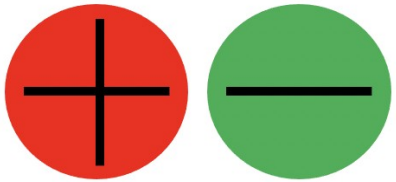
Molecular
PCR (End-Point)
PCR (Real-Time)
Isothermal amplification

Immunoassay
ELFA
ELISA
Lateral Flow

Rapid detection inclusivity

- Inclusivity – What will the assay detect?
  - The target is selected during method development
  - Performance of the target is evaluated during method validation
    - Inclusivity strain set tested on pure cultures at a known CFU/mL
- Inclusivity does not always correlate to method-specific enrichment procedure performance
- Matrix studies evaluate the enrichment procedure
  - *L. monocytogenes, L. innocua* may be the only species included in the matrix study

Detection
(15 min – 4h)



Listeria rapid method inclusivity

- *Listeria* inclusivity - A review of commercially available AOAC method reports
  - The 5 classic *Listeria sensu stricto* – ALWAYS included
  - *L. marthii* – SOMETIMES with newer methods
  - *L. grayi* - SEVERAL methods have added
  - *Listeria sensu lato* (other than *grayi*) – SOME species have been added to SOME methods
    - Typically, only species described before 2018
  - The ”new” *Listeria sensu stricto* – Not included
    - Several kit manufacturers have requested these strains

Listeria inclusivity study data

- **+** positive with all experiments at LOD + 1 $\log_{10}$
- **-** negative with all experiments
- **V** both positive and negative results observed.
- **-*** positive generated at LOD + 2 $\log_{10}$

	Listeria	Real-Time PCR			End-Point PCR	ELFA	
	Method	1	2	3	1	1	2
Sensu stricto	*L. cossartiae* subsp. *cossartiae*	+	+	+	+	+	+
	L. cossartiae subsp. *cayugensis*	+	+	+	+	+	+
	L. cossartiae subsp. *cayugensis*	+	+	+	+	+	+
	L. farberi	+	+	+	+	+	+
	L. immobilis	+	+	+	-	-	-
	L. swaminathanii	+	+	+	-*	+	+
	L. marthii	+	+	+	-*	+	+
Sensu lato	*L. aquatica*	-	-	-	-	-	-
	L. booriae	-	-	-	-	-	-
	L. costaricensis	V	-	-	-	+	+
	L. cornellensis	-	-	-	-	-	-
	L. floridensis	V	-	-	-	-	-
	L. grandensis	V	-	-	-	-	-
	L. newyorkensis	V	-	-	-	-	-
	L. portnoyi	V	-	-	-	-	-
	L. riparia	V	-	-	-	-	-
	L. rustica	V	-	-	-	-	-

Listeria inclusivity study data

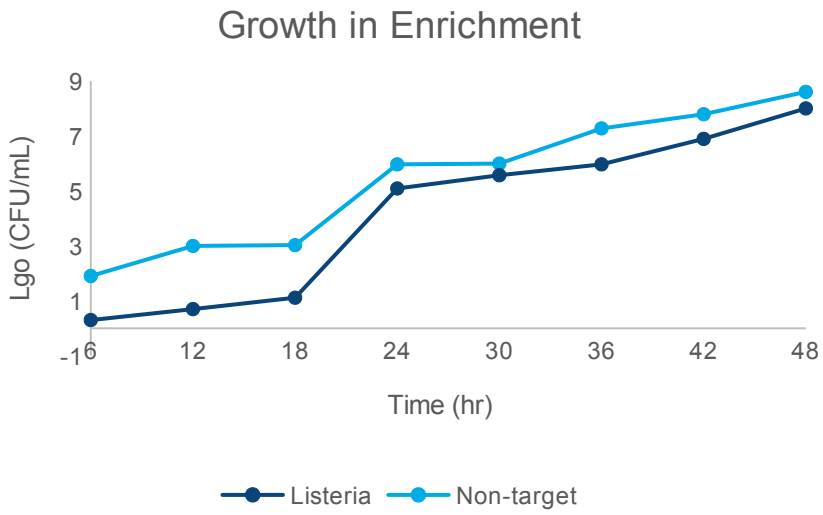
- *Listeria* rapid methods will likely :
  - Be inclusive of “new” *Listeria sensu stricto*
    - Why? *Listeria sensu stricto* show high similarity to each other
    - Example: *L. innocua* clusters closely with *L. farberi*
  - Need to be re-designed for “new” *Listeria sensu lato*
    - Why? *Listeria sensu lato* are dissimilar from *sensu stricto* and each other
    - Example: a method that detects *L. grayi* did not detect “new” *sensu lato*

None of the 6 AOAC methods in the study detected ALL *Listeria* species.

Listeria rapid method – enrichment procedures

- The growth level following enrichment must be at or above the method limit of detection (LOD)
  - Example: 1 CFU/ sponge must grow to 1 x 10^5 CFU/mL to be detected by ELFA
- Enrichment procedure variations include:
  - Single-step or Two-step (i.e., transfer to secondary broth)
  - Incubation time (e.g., 24, 48 h)
  - Incubation temperature (e.g., 30, 35˚C)
  - Selective media formulation

Enrichment (24-48 h)

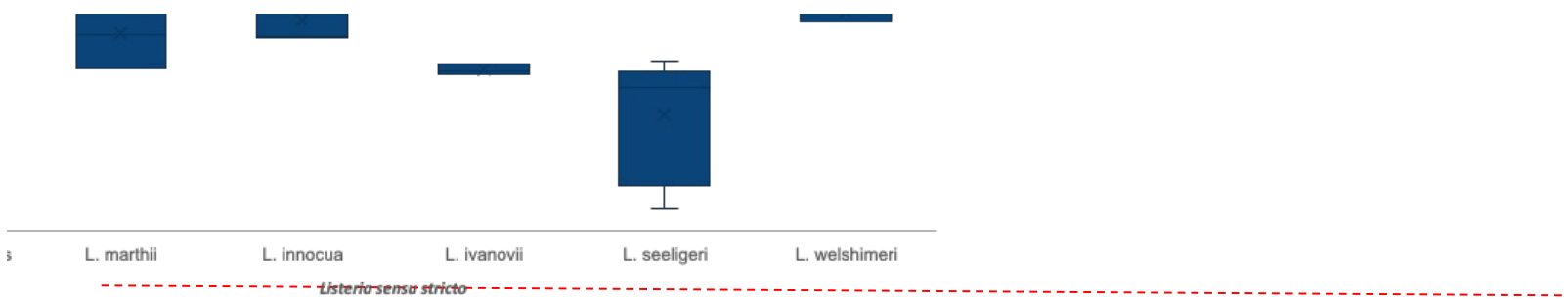


Listeria rapid method – Enrichment procedure

- Published data for enrichment performance is often limited to a few species
- Even when a method is inclusive for *Listeria sensu lato* species, these species may not grow to detectable levels in the enrichment procedure
- Growth studies evaluating larger strains sets have shown:
  - *L. grayi* may not grow to detectable levels
  - Several of the “new” *sensu lato* species will likely not be detected

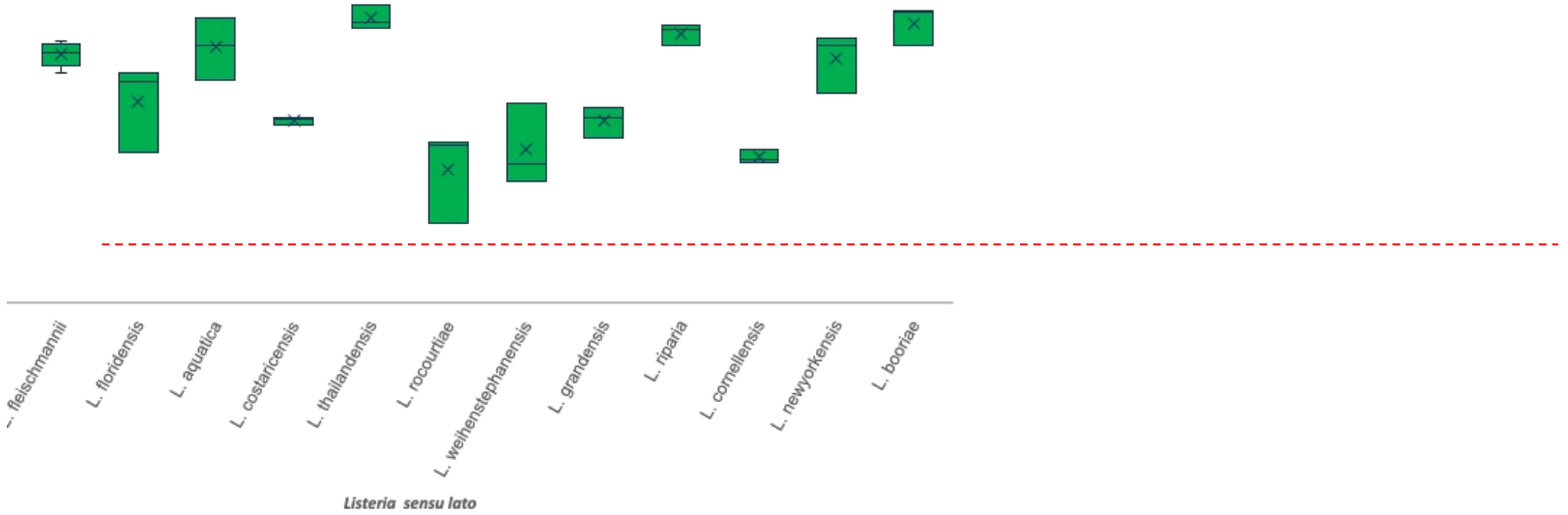
Rapid detection methods – Enrichment Procedures

- A selective broth, incubated at 35˚C, 24 h
  - Selective broth media designed for *L. monocytogenes*
  - Most *Listeria sensu stricto* grew to similar levels as LM
    - *L. ivanovii* and *L. seeligeri* generated lower growth levels than the other *sensu stricto*
  - The red line is the method LOD (4 logs)



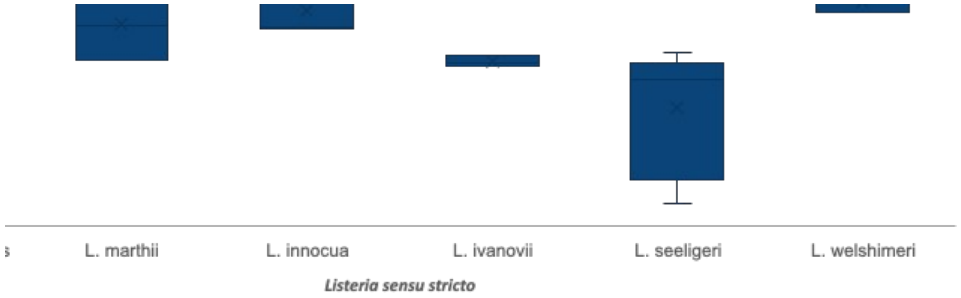
Rapid Detection Methods – Enrichment Procedures

- A selective broth, incubated at 35˚C, 24 h
  - LM growth compared to *Listeria sensu lato*
  - Several *sensu lato* do not grow to detectable levels
- The red line is the method LOD (4 logs)



Rapid detection methods – Enrichment procedures

- What about the 4 new *sensu stricto* species?
  - Current study is focusing on enrichment procedures that are part of ”Next day” detection methods
  - Preliminary study data of 24 h, single-step enrichment procedures is showing:
    - *L. cossartiae, L. farberi, L. swaminathanii* – grew to levels comparable to *L. monocytogenes*
    - *L. immobilis* – grew to levels comparable to *L. seeligeri*, which was often 1-2 logs lower than LM



L. immobilis

Rapid Method Detection - Summary

- *Listeria* spp. rapid detection methods:
- Likely will detect the “new” *Listeria sensu stricto* species
  - End-users should still generate data or confirm this with the manufacturer
  - Real-time PCR methods performed slightly better with respect to detection of *L. immobilis*
- Likely will not detect all the *Listeria sensu lato* species
  - Even if captured in the inclusivity data, growth to detectable levels is dependent on the enrichment procedure
  - This is not concerning when monitoring for the potential for LM contamination

Cultural confirmation

Key points to consider

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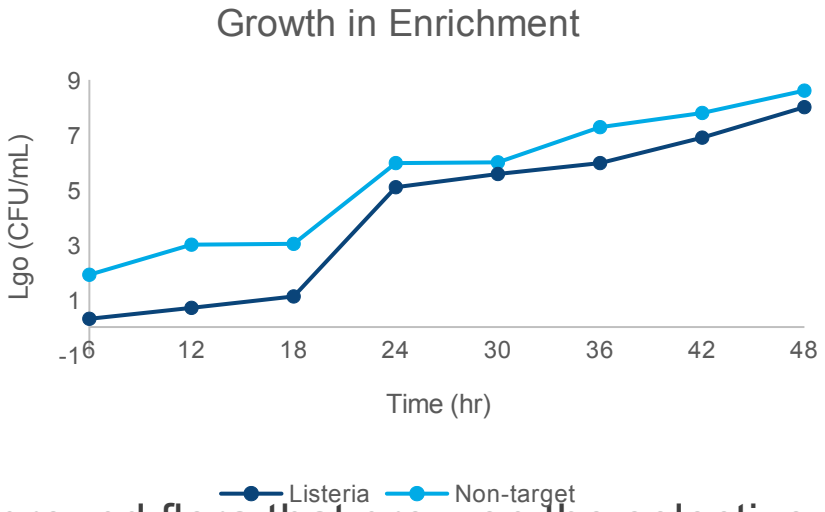
Cultural confirmation

- 3 Main pathways
  1. Genus-level cultural confirmation -Streak at the isolation step
     - Catalase/oxidase – not definitive
     - Complicated by atypical morphologies and potentially high levels of background flora
  2. Species-level confirmation - Select suspect colonies for identification
     - Colony selection can be a challenge
     - Reference methods and database-driven ID systems are not current
  3. If species ID is not desired
     - Don’t pursue cultural isolation

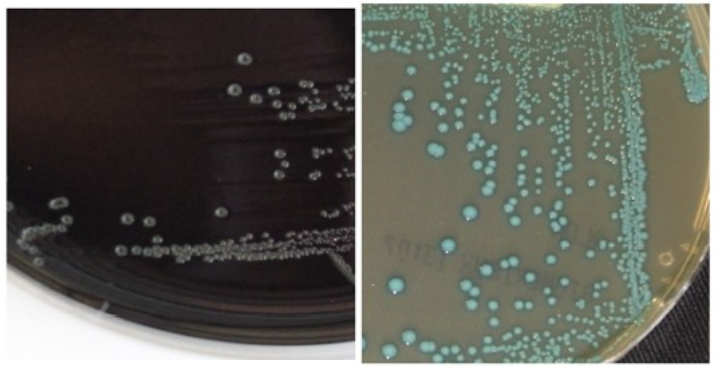
Cultural confirmation



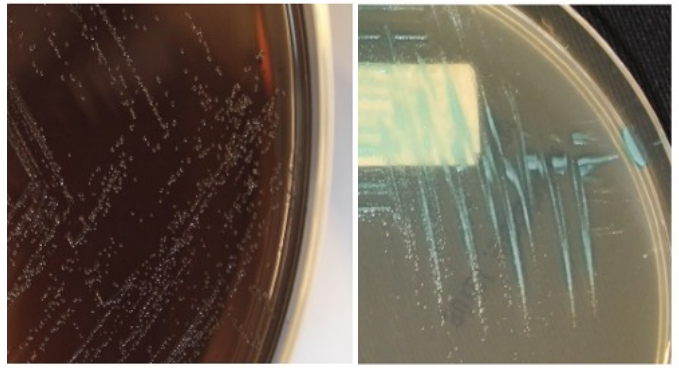
- Consider the impact of the enrichment procedure
  - Less-selective broth media can yield higher levels of background flora that grow on the selective and differential agar
    - Chromogenic agars (e.g., ALOA) have higher specificity than the esculin-based agars (e.g., MOX)
  - *L. seeligeri* and *L. ivanovii* may require extended incubation for cultural isolation
  - Among the *Listeria sensu lato,* several species will grow to comparable levels (or higher) than the *Listeria sensu stricto*
    - Not really an issue if only performing a genus-level confirmation
    - Could complicate species identification

Cultural Confirmation

- IF only concerned with genus-level confirmation
  - Consider atypical colony morphologies may be present
  - Some *Listeria sensu lato* and *L. seeligeri* have shown reduced recovery on selective and differential media



Typical *Listeria* (PI-PLC negative) species



Examples of atypical *Listeria* morphologies

Cultural Confirmation

- IF species identification is required
  1. First, must overcome the challenge of isolating a *Listeria* colony
     - High levels of background flora can make this very difficult
  2. Second, all biochemical schemes and database-driven ID systems have the potential to:
     - Misidentify a “new” *Listeria*
       - “New” *Listeria sensu stricto* will likely be identified as a “classic” *sensu stricto*
     - Generate a ”doubtful profile” or “genus-level ID only” or “*L. ivanovii*”
       - ”New” *Listeria sensu lato*

Cultural Confirmation Summary

- A rapid method suspect that does not confirm culturally is not necessarily a false positive
- "New" *Listeria* can be potentially misidentified → CONSIDER all confirmation data:

Species	Hemolytic Y/N?	PI-PLC Y/N?	ID Result	Questionable?
L. monocytogenes	Y	Y	*L. monocytogenes*	N
L. marthii	N	N	*L. monocytogenes*	Y
L. swaminathanii	N	N	*L. monocytogenes*	Y
L. cossartiae	N	N	*L. monocytogenes*	Y
L. farberi	N	N	*L. innocua*	N*
L immobilis	N	N	*L. ivanovii*	Y**
7 of the "new" *sensu lato*	N	N	*L. ivanovii*	Y**

*A non-pathogenic identified as another non-pathogenic species
**Although the regulations are for LM. *L. ivanovii* has the potential to be pathogenic

Key Takeaways

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Rapid Detection Methods

- Not all *Listeria* species are detected by commercially available rapid detection methods
  - Currently there may be no rapid method that detects all *Listeria*
  - Primarily this is limited to *sensu lato*, which is not a food safety concern
    - There is strong evidence to support *Listeria sensu lato* (e.g., *L. grayi, L. rocourtiae*) should not be classified as *Listeria*
  - Detecting a *sensu lato* is not bad, but does not appear necessary for food safety
- End-users should assess rapid detection methods for *Listeria sensu stricto* detection

Cultural confirmation

- *Listeria* spp. cultural isolation can be challenging
  - " New" *Listeria* database representation is poor
    - The new *sensu stricto* will likely be misidentified, in some cases as LM
    - Very important to consider hemolysis and PI-PLC activity, DIM is also helpful
    - "New" *Listeria* are not defined in the reference methods
  - Isolating the organism that generated the rapid method positive may be a "needle-in-a-haystack"
  - If identifying LM is the goal, run a secondary screen using an LM assay
    - Most manufacturers have an LM assay in their catalogue that uses the same enrichment procedure
  - If not concerned with species ID - is confirmation necessary?

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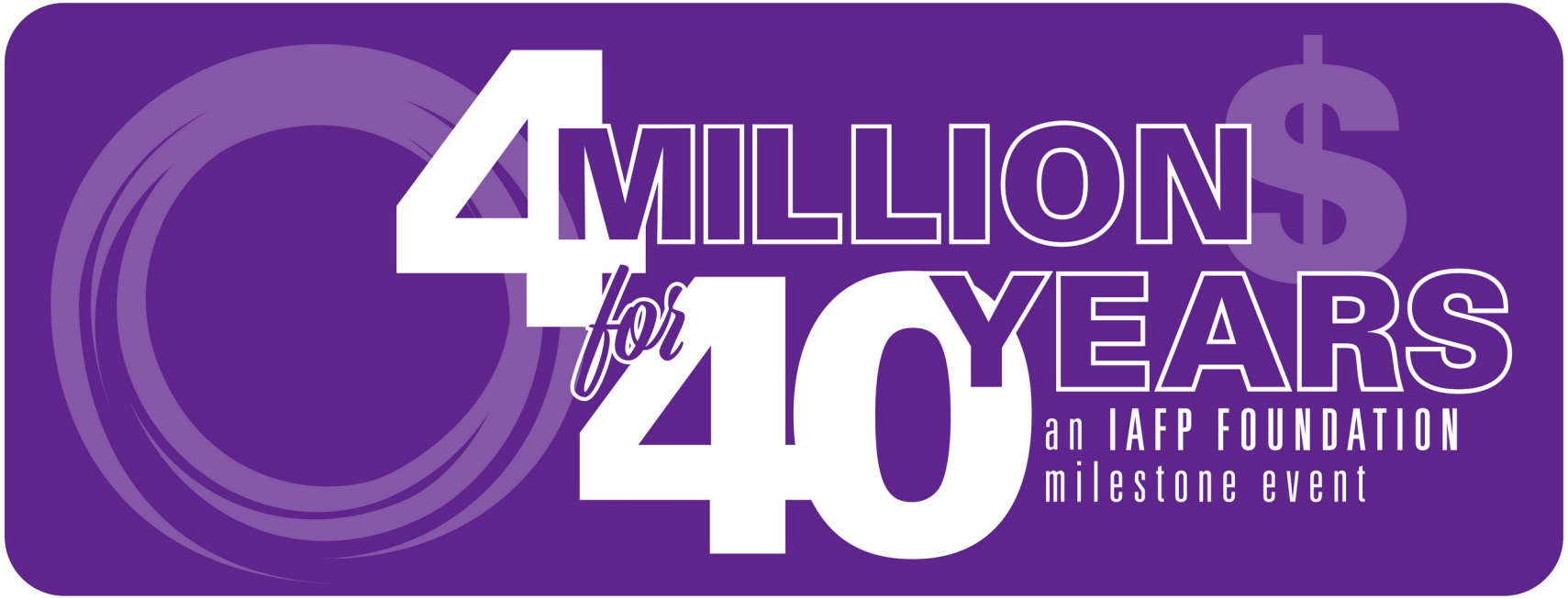

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