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10 years of organizing *L. monocytogenes* enumeration proficiency testing in food matrices at the Belgian national reference laboratory

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Listeria monocytogenes is a common low-level contaminant in ready-to-eat foods of both plant and animal origin. It causes listeriosis, a disease with the highest hospitalization and fatality rates among foodborne pathogens. In 2009, the Belgian food competent authority (FASFC) has designated Sciensano as national reference laboratory (NRL) for *L. monocytogenes*. One of the NRL's task is to organize proficiency tests (PT) for official laboratories and inform the competent authority about the reliability of the participant's results. Sensitivity, specificity and the overall performance of the network were assessed on the 2014 – 2023 period.

"Organizing PT's is one of the tasks of the NRL"
article 101 EU Regulation 2017-625

Method

☑ Enumeration of *L. monocytogenes*

☑ Participants: Belgian official laboratories

☑ Frequency: once a year, start at a fixed date

☑ One different food matrix each year

☑ Methods used by the FASFC

☐ Challenges:
- real food matrices with natural background flora
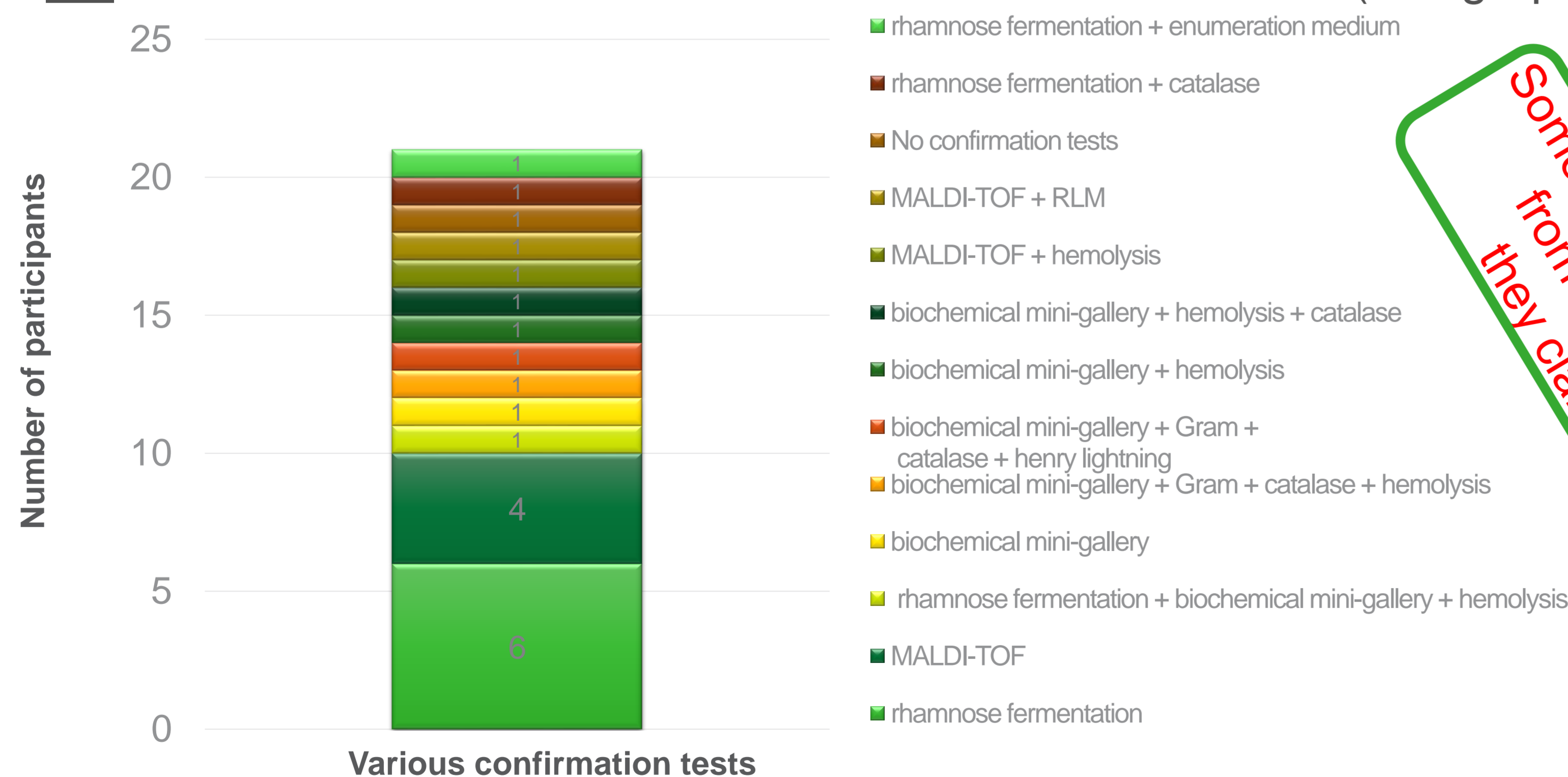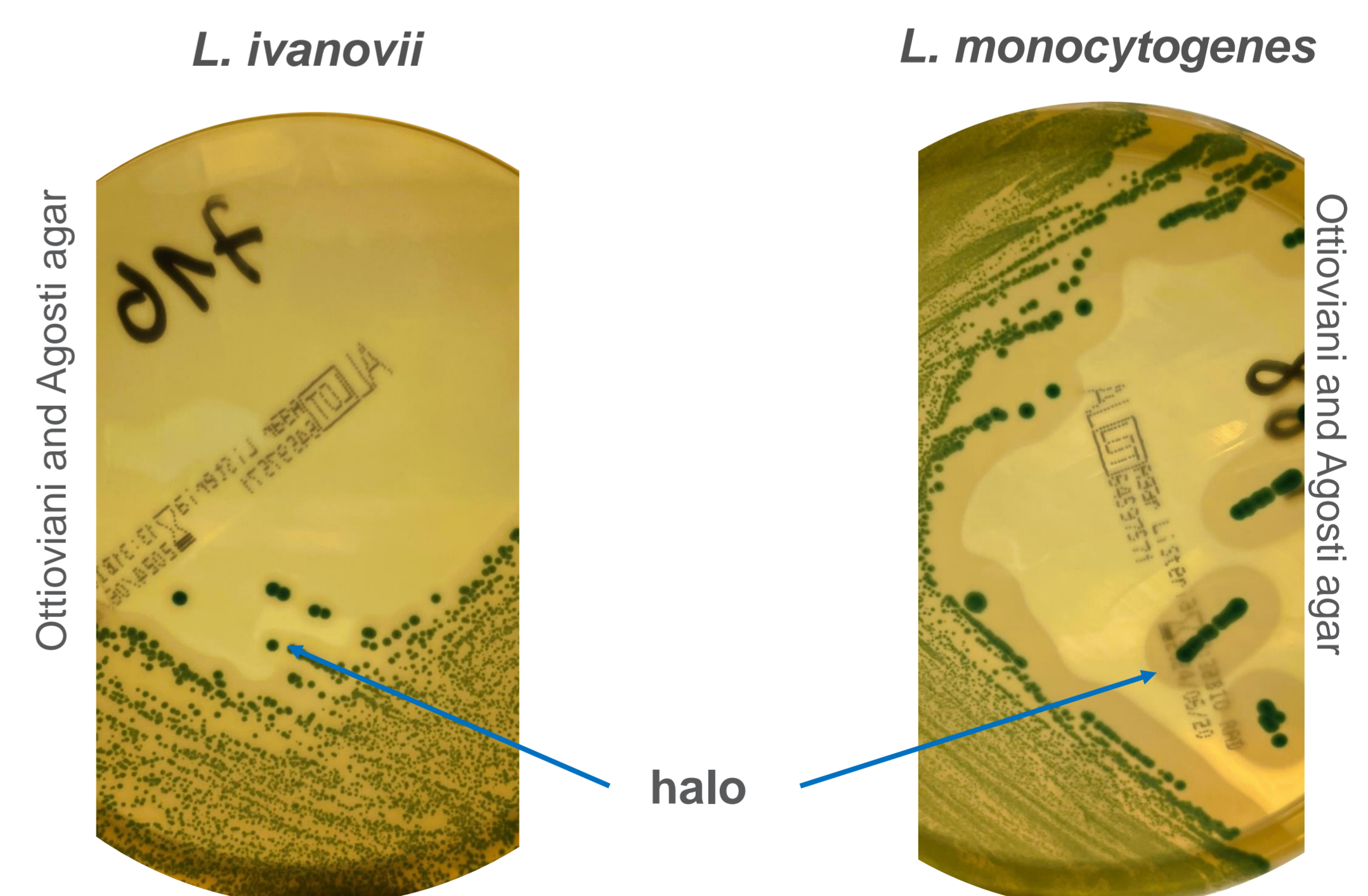- some samples spiked with *L. ivanovii* instead of *L. monocytogenes*

Discussion

• Methods used by the participants:

→ two main **enumeration media** :
- Ottioviani and Agosti agar, required by the EN/ISO 11290-2 enumeration method
- one alternative ISO 16140-2 validated medium

→ but various **confirmation tests** or even no confirmation test (see graphic)

Some laboratories deviate from the method they claim for

legend (confirmation tests):
- rhamnose fermentation + enumeration medium
- rhamnose fermentation + catalase
- No confirmation tests
- MALDI-TOF + RLM
- MALDI-TOF + hemolysis
- biochemical mini-gallery + hemolysis + catalase
- biochemical mini-gallery + hemolysis
- biochemical mini-gallery + Gram + catalase + henry lightning
- biochemical mini-gallery + Gram + catalase + hemolysis
- biochemical mini-gallery
- rhamnose fermentation + biochemical mini-gallery + hemolysis
- MALDI-TOF
- rhamnose fermentation

Various confirmation tests

• Performance of the participants is expressed using **z-score** based on the consensus value from participants and the variability of the reported results.

Results

Performance of the laboratories

	Sensitivity	Specificity
contaminated samples (581)	100%	/
blank samples not spiked with *L. ivanovii* (182)	/	99.4%
blank samples spiked with *L. ivanovii* (268)	/	86.9%

Decrease due to false positive results

False-positive results samples spiked with *L. ivanovii* (%)

Year	Value
2015	10
2016	19
2017	24
2018	15
2019	5
2020	24
2021	15
2022	2
2023	0

L. ivanovii — Ottioviani and Agosti agar

L. monocytogenes — Ottioviani and Agosti agar

halo

Conclusion

Combining all results, the accuracy of the network is estimated at 96.5%, which is considered good. However, for samples contaminated with *L. ivanovii*, successive rounds do not show stable positive progress. Depending on the enumeration medium, confirmation tests are essential to distinguish between *L. monocytogenes* and *L. ivanovii*. The sensitivity is excellent and stands at 100%. Concerning the variability of the results of positive samples, it remains stable overtime.

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