

Performance Evaluation of Easy Plate™ LS for Selective Detection of *Listeria* Species

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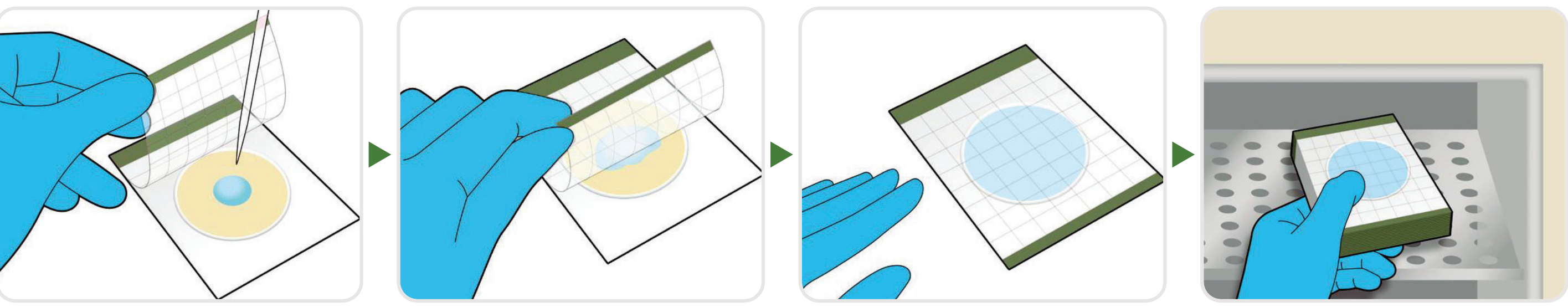
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Introduction

- *Listeria monocytogenes* is a major foodborne pathogen causing listeriosis through contaminated food. Environmental monitoring for *Listeria* species is important for controlling *L. monocytogenes*.
- The **Easy Plate** series are Ready-to-Use (RTU) media developed and manufactured by Kikkoman Biochemifa Company, and currently comprise seven products: AC, AC-R, CC, EC, EB, SA, and YM-R. Compared to conventional plate media, Easy Plate offers several advantages, including **reduced testing time, ease of operation, space efficiency**, and **lower plastic usage**.
- The newly developed ready-to-use (RTU) media for *Listeria* species, **Easy Plate LS (E-LS)** are convenient and environmentally friendly and allow for rapid enumeration in just 24 hours.
- This study evaluated the selectivity of E-LS, conventional agar media (ALOA, PALCAM), and other RTU media (RTU-A, B) using *Listeria* strains and non-target strains, including food-derived isolates.

How to use Easy Plate



Count blue colonies

Methods

For the inclusivity study, 32 *L. monocytogenes* strains and 28 strains of other *Listeria* spp. were tested. For the exclusivity study, 30 non-target strains were tested. Each strain was enriched overnight in TSB or YM broth and diluted in BPW. E-LS was compared with two conventional agar media (ALOA and PALCAM agar) and two RTU media (RTU-A and RTU-B). Conventional agar media were inoculated by spread plating method, whereas RTU-A and RTU-B were tested according to their instructions for use. *Listeria* strains were inoculated at approximately 100 CFU per plate, whereas non-target strains were inoculated at $\geq 10^3$ CFU per plate. E-LS was incubated at 35 and 37°C, while all comparator media were incubated at 37°C. Colony growth was assessed after 24, 28, and 48 h.

Results & Discussion

The numbers of strains showing positive results are summarized in the tables. In the inclusivity study, E-LS detected **92% (55/60)** of the tested strains, including all *L. monocytogenes* strains, after 24 h of incubation. In particular, the growth of *L. seeligeri* and *L. grayi*, which are often slow-growing and difficult to detect, was detected earlier on E-LS than on the other media tested. Some strains showed weaker growth at 37°C than at 35°C, as indicated by lower colony counts and smaller colony sizes.

Table 1. The result of **Inclusivity** study (*Listeria* spp.)

Strain		E-LS 35°C			E-LS 37°C			ALOA		PALCAM		RTU-A			RTU-B	
Incubation time [h]		24	28	48	24	28	48	24	48	24	48	24	28	48	24	48
<i>L. monocytogenes</i>	32	32	32	32	32	32	32	32	32	31	32	31	31	31	21	28
<i>L. innocua</i>	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7
<i>L. ivanovii</i>	4	2	3	4	2	3	4	4	4	2	4	0	0	0	0	3
<i>L. seeligeri</i>	4	3	3	3	2	2	3	0	2	0	2	0	0	0	0	0
<i>L. welshimeri</i>	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
<i>L. grayi</i>	4	2	3	4	2	3	4	3	3	2	2	0	0	2	1	1
Other <i>Listeria</i> spp. ⁽¹⁾	5	5	5	5	5	5	5	5	5	3	5	4	4	4	2	3
Total	60	55	57	59	54	56	59	55	57	49	56	46	46	48	33	46
Total [%]	100	92	95	98	90	93	98	92	95	82	93	77	77	80	55	77

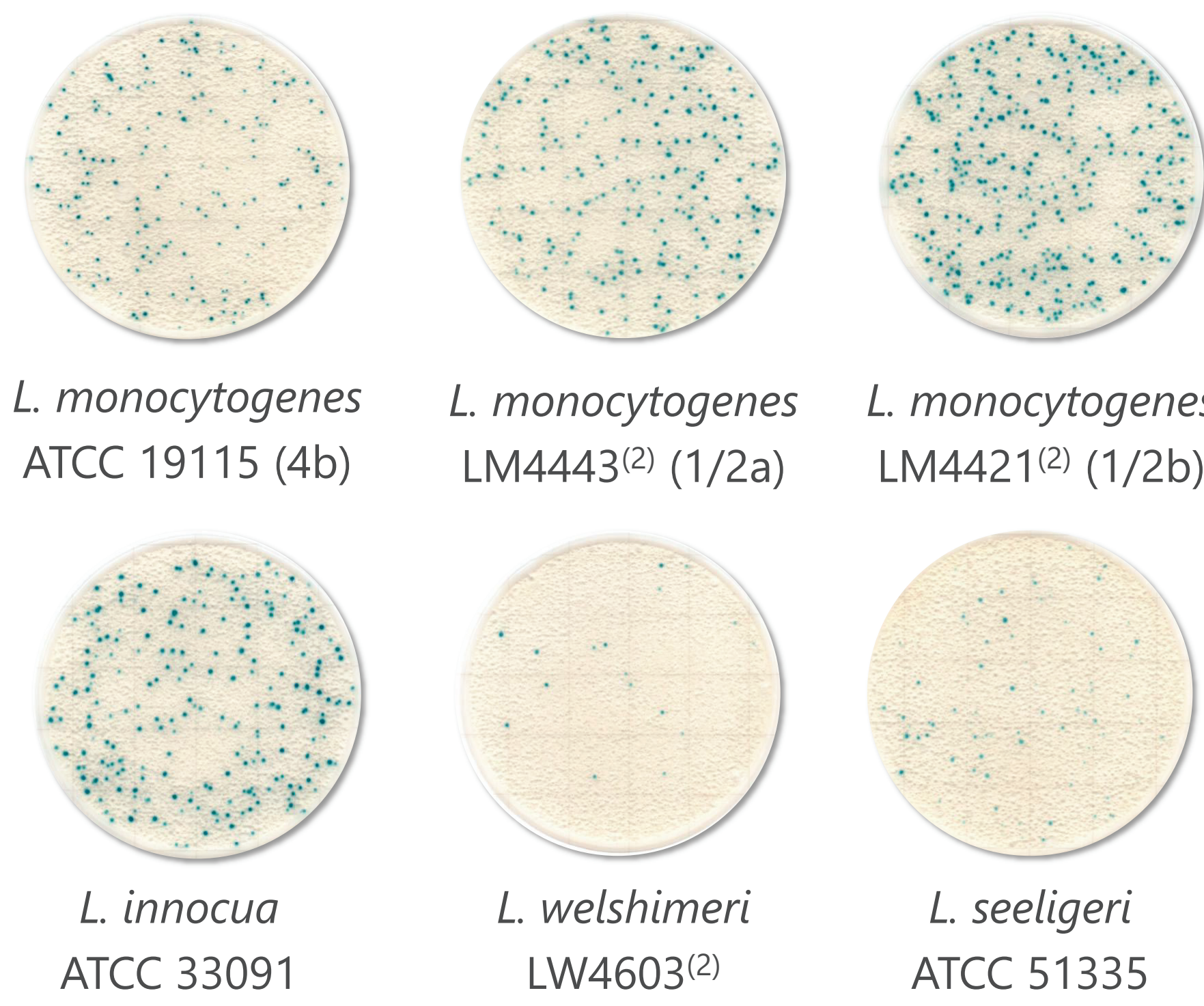
(1) *L. cossartiae* (2 strains), *L. farberii*, *L. immobilis*, *L. marthii* (all belonging to *Listeria* sensu stricto)

In the exclusivity study, prolonged incubation promoted the growth of some Gram-positive bacteria, including *Enterococcus*, lactic acid bacteria, and *Bacillus* spp., on comparator media. In contrast, all of these organisms were inhibited on E-LS after 24 h of incubation.

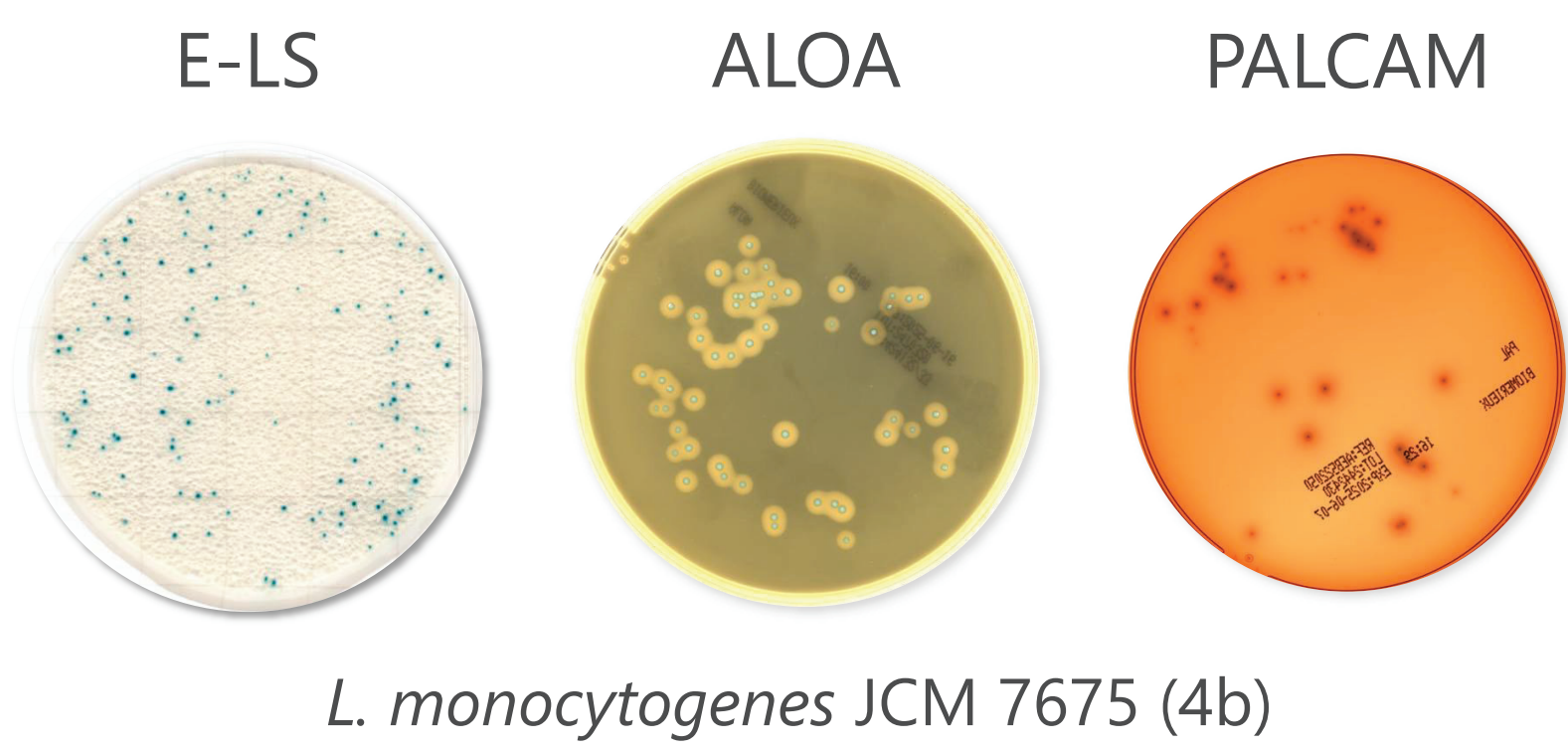
Table 2. The result of **Exclusivity** study (non-target strains)

Strain		E-LS 35°C			E-LS 37°C			ALOA		PALCAM		RTU-A			RTU-B	
Incubation time [h]		24	28	48	24	28	48	24	48	24	48	24	28	48	24	48
Gram positive	20	0	0	0	0	0	2	0	0	2	2	0	0	0	1	3
Gram negative	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yeast and mold	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Images after 24 h incubation



(2) Culture collection of Food Safety Research Center, Tokyo University of Agriculture



Conclusion

E-LS shows high selectivity and reliable detection of most *Listeria* species within 24 hours. By removing complex preparation steps, this medium is expected to significantly enhance environmental monitoring programs for *Listeria*.

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