3M™ Petrifilm™ Environmental Listeria Plate

Keeping *Listeria* out of the food-manufacturing environment is the best defense against product contamination. A strong environmental monitoring program is a vital preventative measure in the ongoing management of this microorganism. The 3M™ Petrifilm™ Environmental Listeria Plate was developed to provide rapid and quantitative *Listeria* results from environmental samples.

The performance of the 3M™ Petrifilm™ Environmental Listeria (EL) Plate method was evaluated for:

I. **Sensitivity and specificity:** growth comparison of 88 pure strains using the Petrifilm EL Plate method and the reference ISO method [ISO 11290-2: 1998(E)]*

Sensitivity is defined as the ability of the method to detect the target organism *if present*, compared to the reference method.

Specificity is defined as the ability of the method to detect *only* the target organism, and not non-target organisms, compared to the reference method.

*International Standard ISO 11290-2: 1998(E) "Microbiology of Food and Animal Feeding Stuffs —Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method." Available at http://www.iso.org

II. **Quantification:** colony count comparison of pure strains tested using the Petrifilm EL Plate method and the ISO method

III. **Validation:** external studies performed at independent plant site laboratories

Summary:

Qualitative Results

Sensitivity = 98% compared to the ISO method
Specificity = 100% compared to the ISO method

Quantitative Results

- No statistical difference from ISO method
- No lab-to-lab variability

3M Microbiology

Experiments

Test Organisms

Pure bacterial cultures were derived from lyophilized preparations purchased from the American Type Culture Collection or from frozen stock cultures of isolates that had been identified by biochemical methods. Fifty-one *Listeria* isolates (Appendix 1) and thirty-seven non-*Listeria* isolates (Appendix 2) were tested.

Study Design

Cultures were grown in trypticase soy broth, and then analyzed using the reference ISO method [ISO 11290-2: 1998(E)] and the Petrifilm EL Plate method (see product package insert for details). Briefly, the cultures were diluted, mixed with buffered peptone water, allowed to stand for one hour to encourage cell repair, and then dispensed onto a Petrifilm EL Plate (3 mL). The sample was also spread-plated (1 mL) onto duplicate 150-millimeter polymyxin-acriflavin-ceftazidime-lithium chloride (PALCAM) agar plates for the ISO method. The Petrifilm EL Plates were incubated for 28 hours at 35ºC. The PALCAM agar plates were incubated for 48 hours at 35ºC according to ISO 11290-2 and other standard methodologies. Formation of typical colonies on each media type was noted as positive or negative, and counts were recorded.

Data Analyses

The colony counts from each of the PALCAM agar plates were multiplied by three and then were averaged following the formula included in the ISO method. To compare counts, the average PALCAM counts and the Petrifilm Plate counts were converted to log base 10 and a paired t-test was performed. The sensitivity and the specificity were calculated for the Petrifilm EL Plate method against the ISO method (Table 1) using the 88 organisms tested in this study (Appendices 1 and 2).

Results

I. Sensitivity and Specificity

Table 1. Identification of *Listeria* by Petrifilm EL Plate method versus ISO method.

		ISO 11290-2 Method +	ISO 11290-2 Method -
Petrifilm Plate Method	+	46	0
	-	1	37

Where: Sensitivity = 46/(46+1) False Negative Rate = 1/(46+1)
Specificity = 37/(0+37) False Positive Rate = 0/(0+37)

Sensitivity = 98%	**False Negative Rate = 2%**
Specificity = 100%	**False Positive Rate = 0%**

Results *(continued)*

II. Enumeration / Quantification

Table 2 shows the results of a comparison of colony counts from the Petrifilm EL Plate method and from the ISO 11290-2 method. By analysis of variance, the mean log counts of *Listeria* were not statistically different between the methods ($p > 0.05$).

Table 2. Results of comparison of the Petrifilm EL Plate method to ISO method for the enumeration of *Listeria*.

Number of samples	Mean log difference	Standard error	t value	p value
46	0.04	0.02	1.94	0.06

III. External Validation Study

Seven different samples comprised of *Listeria* (target organism), *Bacillus* and/or *Enterococcus* (non-target organisms) species were prepared by an outside reference laboratory. Replicate samples were shipped to four independant plant site laboratories where they were analyzed by multiple technicians using the Petrifilm EL Plate method. Within-sample variation showed agreement 99% of the time, and variation between technicians showed agreement 98% of the time.

Conclusions

Sensitivity and specificity of the Petrifilm EL Plate method were 98% and 100%, respectively, in comparison to the ISO 11290-2 method.

Quantitative results of the Petrifilm EL Plate method were not statistically different from quantitative results of the ISO method ($p > 0.05$), and no lab-to-lab variability was determined in the external validation study.

The Petrifilm EL Plate method provided quantitative and qualitative results equivalent to the ISO method, and is a convenient, sample-ready method that increases labor productivity and provides powerful information for efficient environmental monitoring.

Appendix 1

The 51 *Listeria* strains tested and the sources from which they were isolated are as follows:

Listeria monocytogenes:
bovine – J2-031
cheese – ATCC 51772
chicken – ATCC 19116, ATCC 19118
cow – J2-020, J2-064, RT-54
derived from Scott A – ATCC 49594
food epidemic – J1-110
goat – J1-158, J2-035
human – ATCC 19113, ATCC 19115, C1-056, C1-115, C1-122, J1-031, J1-049, J1-094, J1-168, J1-169, J1-177, J1-225, M1-004, N1-225
Mexican-style cheese – ATCC 43256, ATCC 43257
poultry – ATCC 19111, RT-1637
sheep – ATCC 19117, J2-054, J2-063, J2-066;
spinal fluid – ATCC 19112
unknown – ATCC 19114, W1-110, W1-111, X1-010, RT-472
raw milk – ATCC 51414

Listeria innocua:
cabbage – ATCC 51742
cow brain – ATCC 33090
derived from 33090 – ATCC 49595
human feces – ATCC 33091
unknown – Li2236, Li2248

Listeria grayi:
unknown – ATCC 700545

Listeria seeligeri:
unknown – ATCC 35967

Listeria ivanovii:
sheep – ATCC 19119

Listeria welshimeri:
decaying plant material – ATCC 35897
unknown – RT-7233

Appendix 2

The 37 non-*Listeria* strains tested are as follows:

Bacillus cereus – ATCC 13061
Bacillus circulans – ATCC 61
Bacillus coagulans – ATCC 7050, ATCC 23498
Bacillus pumilus – ATCC 72, A-6
Bacillus subtilus – ATCC 6051, ATCC 23856, ATCC 29056
Brevibacterium linens – ATCC 9172
Enterococcus faecalis – ATCC 6055, ATCC 7080, ATCC 14506, ATCC 29212
Enterococcus faecium – ATCC 882, ATCC 12952, ATCC 35667, ATCC 49624
Erysipelothrix rhusiopathiae – ATCC 19414
Escherichia coli ATCC 33456
Kurthia zopfii – ATCC 6900
Lactobacillus alimentarius – ATCC 29643
Lactobacillus brevis – Bbr
Lactobacillus farciminis – ATCC 29644
Lactobacillus fermentum – ATCC 9338
Lactobacillus johnsonii – ATCC 11506
Lactobacillus plantarum – ATCC 49445
Lactococcus lactis subsp. *cremoris* – ATCC 9596
Lactococcus lactis subsp. *lactis* – ATCC 19435
Pediococccus acidilacrici – PA
Pediococcus pentosaceus – PP
Pseudomonas fragi – ATCC 51821
Staphylococcus aureus – ATCC 25923
Streptococcus bovis – P89
Streptococcus mutans – ATCC 25175
Streptococcus sanguis – ATCC 10556
Streptococcus viridans – M1040

3M

3M Microbiology
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1-800-228-3957
microbiology@mmm.com
www.3M.com/microbiology

3M Canada
Post Office Box 5757
London, Ontario N6A4T1
Canada
1-800-563-2921

3M Europe
Laboratoires 3M Santé
Boulevard de l'Oise
95029 Cergy Pontoise
Cedex
France
33-1-30-31-8571

3M Latin America
Avenida Santa Fe 55, Santa Fe
C.P. 01210 Mexico City
Mexico
5255-5270-0454

3M Asia Pacific
9 Tagore Lane
Singapore 787472
65-64548611

3M Australia/New Zealand
9 - 15 Chilvers Road
Thornleigh, NSW 2120
Australia
1300 363 878

3M Japan
31-1, Tamagaradai,
2-Chome
Setagaya-Ku, Tokyo
158-8583, Japan
81-3-3709-8289

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70-2009-6385-1