The rapid detection of listeria from environmental samples

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Bacteria belonging to the Listeria family are Gram positive, non-spore forming, rod shaped organisms and they are capable of surviving in food processing plant environments.

Listeria bacteria are relatively thermo-tolerant which means they are able to survive at both refrigerated and high temperatures. Furthermore, they have been found to grow in high salt concentrations and over a wide pH range.

Substantial risk

The organism is able to form biofilms on environmental surfaces and can remain on the surface of a processing plant for years. Blackman et al found that the biofilm formation of listeria on stainless steel was sufficient to provide a substantial risk of contamination of the food processing environment that was studied in this experiment.

Listeria spp. are often used as indicator organisms especially in food processing plants making products that are particularly susceptible to listeria contamination and incubation.

The presence of the non-pathogenic species of this genus is indicative of poor sanitation practices and the possible presence of the pathogenic Listeria monocytogenes.

Outbreaks of pathogenic Listeria monocytogenes have often been traced back to contamination of the same strain on equipment in the processing plant. Common surfaces where contamination can occur include filling equipment, packing equipment, conveyer belts, product transport racks, hand tools, gloves, freezers, air and floor vents, and gaskets.

Current methods for testing for listeria are time consuming and laborious. The development of test media specific to the growth and identifying of the Listeria genus has resulted in a chromogenic media that has been demonstrated to reduce time to results with increased ease of use.

Species	Serotypes tested	No. of strains tested	With blue colonies	With white colonies
Bacillus species	16	16	0	0
Enterococcus species	10	9	1 (light – atypical)	0
Lactic bacteria (Lactobacillus, Lactococcus, Pediococcus, Propionibacterium)	16	16	0	0
Other Gram positive bacteria (Brochotrix, Carnobacterium, Corynebacterium, Erysipelothrix, Jonesia, Kurthia, Micrococcus, Rhodococcus, Staphylococcus, Streptococcus)	26	15	0	2
Gram negative bacteria (E. coli, Citrobacter, Enterobacter, Klebsiella, Proteus, Salmonella, Pseudomonas)	13	13	0	0
Yeasts	2	1	0	1

Table 2. Exclusivity study.

Table 1. Inclusivity study.

Species	Serotypes tested	No. of strains tested	With blue colonies	With white colonies
Listeria monocytogenes	1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4d, 4d, 4e, 7	24	24	0
Listeria innocua	6a, 6b	6	6	0
Listeria ivanovii	5	5	5	0
Listeria welshimeri	6a, 6b	7	7	0
Listeria seeligeri	1/2b	6	6	0
Listeria grayi		3	2	1

Chromogenic medium

The Bio-Rad media RAPID'Listeria spp. is a chromogenic medium for isolation and detection of Listeria spp. from environmental surfaces.

It is a selective agar which combines chromogenic substrates and biochemical indicators that reveal Listeria spp. It achieves this by combining the action of lithium chloride and an antibiotic mixture that allows listeria to survive and grow.

By using a chromogenic substrate to detect ß-D-glucosidase activity all listeria

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Surface	Level	Inoculation	No. samples	RAPID' Listeria spp. positive	Reference positive	Method agreement	X^2
Stainless steel (internal)	Control Low	0 3.1x103	5 20	0 19	0 19	100% 100%	- -
Stainless steel (independent)	Control Low	0 2.7x102	5 20	0 19	0 19	100% 100%	- -
Plastic	Control Low	0 2.3x103	5 20	0 8	0 8	100% 100%	- -
Ceramic	Control Low	0 2.3x103	5 20	0 5	0 8	100% 85%	- 1.33
Sealed concrete	Control Low	0 8.7x102	5 20	0 17	0 19	100% 90%	- 0.50

Table 3. Method comparison study results.

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colonies are seen as blue to blue-green. The objective of the research was to find out if RAPID'Listeria spp was capable of accurately identifying the target organism in samples taken from a variety of environmental surfaces, and able to demonstrate a level of inclusivity and exclusivity comparable to the USDA/FSIS MLG reference method.

Listeria isolates

For the inclusivity and exclusivity studies 51 food and clinical isolates of Listeria spp. and 83 non-listeria organisms were tested on RAPID'Listeria spp. Listeria strains were cultured in University of Vermont Medium (UVM) at 30°C for 18-24 hours.

Non-listeria strains were cultured overnight in nutrient broth. Cultures were streaked for isolation on RAPID'Listeria spp. agar and incubated at 37°C for 24 hours.

Of the 51 listeria strains tested on RAPID'Listeria spp. 50 strains produced typical blue colonies. One strain of Listeria grayi produced small, white atypical colonies.

When the incubation of this plate was continued, the strain produced a typical blue colony after 48 hours. Two other L. grayi strains were tested and produced typical blue colonies after 24 hours.

The inclusivity rate was calculated as 98%. Some 83 non-listeria organisms were tested on RAPID'Listeria spp. agar for the exclusivity study. Only four were able to grow and none of these organisms produced typical blue colonies. The exclusivity rate was calculated as 100%.

Comparison study

The purpose of the method comparison study was to measure the performance of RAPID'Listeria spp. against the USDA/FSIS MLG reference method for detection of Listeria spp. from environmental surfaces.

Four surfaces were selected – stainless steel, plastic, ceramic, and sealed concrete.

Stainless steel was processed a second time as part of the independent laboratory validation.

One set of primary enrichment broth samples (UVM) was prepared for the reference method and for the RAPID'Listeria spp. method.

After 24 hours incubation, a 0.1ml aliquot of UVM was spread across half of one pre-dried RAPID'Listeria spp. plate using a sterile swab. An inoculating loop was used to streak for isolated colonies. Plates were incubated at 37°C ± 1°C for 24 hours.

Typical blue to blue-green colonies were selected and confirmed according to the USDA/FSIS MLG reference method.

The proportion of positive samples for the reference method was compared to the proportion of positive samples for RAPID'-Listeria spp. method using McNemar's Chi square method for paired analysis. A X2 value of less than 3.84 was indicative of no significant difference at the 5% probability level.

Method agreement was also calculated and expressed in terms of percentage.

Overall method agreement was calculated by determining a weighted average of all environmental surfaces.

Method agreement for plastic and both the internal and independent stainless steel surfaces was 100%.

No significant difference was observed between the RAPID'Listeria spp. method and reference method for ceramic and sealed concrete.

Conclusions

It can be concluded, therefore, that RAPID'Listeria spp. agar allows for isolation and identification of Listeria spp. from environmental surfaces and was able to correctly identify 98% of the listeria strains tested in the inclusivity study and 100% of the non-listeria strains tested in the exclusivity study.

When compared to USDA/FSIS MLG reference method, RAPID'Listeria spp. agar was shown to be an effective and efficient alternative medium for detection of Listeria spp. from stainless steel, plastic, ceramic, and sealed concrete, with an overall method agreement of 96%.

A shortened enrichment protocol (24 hours) was validated against the reference method (48 hours). ■

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