A Research Note

PERFORMANCE OF THE ALOA™ MEDIUM IN THE DETECTION OF HEMOLYTIC *LISTERIA* SPECIES IN FOODS AND ENVIRONMENTAL SAMPLES

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ABSTRACT

As an addition to the standard plating media the selective differential ALOA ™ *Listeria* plate was evaluated for the detection of hemolytic *Listeria* species in a variety of food and environmental samples.

***Listeria* cells in pre-enrichment broths were plated onto the *Listeria* ALOA™ medium, and routine indicator media (Oxford and PALCAM agar). 153 of the 566 preenrichment samples tested were positive for hemolytic *Listeria* species using both the ALOA ™ medium and the standard culture technique. A hemolytic *Listeria* positive on the ALOA agar plate results in a blue colony (presence of X-glucoside) with a halo (metabolism of an unspecified substrate by phospholipase). The specificity of the ALOA ™ technique was studied using 38 pure cultures of different *Listeria* species and non-*Listeria* Gram positive and Gram negative bacteria.**

Compared to the standard Health Canada culture method, which utilizes selective plating on Oxford and PALCAM agar, the ALOA ™ technique showed 100% specificity, 100% sensitivity, a false negative rate of 0% and a false positive rate of 0% in the analysis of naturally contaminated and spiked foods and environmental samples for hemolytic *Listeria* (*Listeria monocytogenes* and *L. ivanovii*).

INTRODUCTION

Listeria monocytogenes **has been found increasingly as implicated in food borne illness in Canada and the United States over the past few years. The food products most commonly identified as vehicles for transmission of** ***Listeria*** **include hot dogs, ready-to-eat (RTE) meat products, fresh fruit and vegetables. The agencies responsible for reporting of listeriosis, and other food borne illnesses (United States Centre for Disease Control (CDC), Health Canada (LCDC), Canadian Food Inspection Agency (CFIA, Office of Food Safety and Recall), state and provincial health units, etc), have become more active in attempting to control, reduce and eliminate** ***Listeria*** **from foods and the environment. As the cost to the health system, private sector and individuals increases there is a need for more rapid, accurate screening tests for the detection of** ***Listeria*** **from foods, food sources and the environment (Sharpe, 1994; Vasavada, 1993).**

The ALOA™ ***Listeria*** **technique (AES Laboratories, France) was designed as a rapid selective and differential plating medium for the identification and isolation of hemolytic** ***Listeria*** **species, notably;** ***Listeria monocytogenes*****, from selective enrichment cultures. Selectivity is accomplished through the addition of lithium chloride and an antimicrobial mixture (unspecified by AES). Differentiation is achieved using the chromogenic compound X-glucoside as a substrate for the detection of beta-glucosidase enzyme, common to all** ***Listeria*** **species (blue coloured colonies). Specificity is the result of the metabolism of a substrate (not described by AES) by phospholipase, an enzyme present in** ***L. monocytogenes*** **(and some** ***L. ivanovii*****), producing a halo around the blue colonies.**

In this study we evaluated the performance of the ALOA ™ ***Listeria*** **plating medium as an additional selective indicator agar media compared to the standard culture medium, Oxford and PALCAM agar plates, in the routine analysis of** ***Listeria*****.**

MATERIALS AND METHODS

Bacteria

A variety of *Listeria* species were used in this study from the Laboratory Services Division Culture Collection (LSDCC) as well as pure cultures from the American Type Culture Collection (ATCC), including: *L. monocytogenes* (serotypes; 1c, 1/2a, 1/2b, 2, 3a, 3b, 4b, 4bx, 4c, 4d, 4e and 7), *L. ivanovii* (ATCC # 19119), *L. innocua* (ATCC # 33090, serotype; 4ab) and *L. seeligeri* (LSDCC # 528, serotype; 4a).

Unless otherwise stated, cultures were routinely grow by shaking in nutrient broth (NB, Difco) at 35-37°C for 16-20h. Whenever necessary, viable counts were obtained by plating serial dilutions of broth cultures on nutrient agar (NA, Difco) and trypticase soy agar with 0.5% yeast extract (TSA-YE, Difco) and incubating the plates at 35 -37°C for 16-20h. In addition, pure cultures of a variety of non -*Listeria* Gram positive and Gram negative bacteria were also examined, including: *Bacillus cereus, B. subtilis, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Lactobacillus casei, Proteus vulgaris, Pseudomonas aerogino sa, Salmonella agona, S. enteritidis, S. typhimurim, S. mbandaka, S. senftenberg, Serratia marcesens, Shigella sonnei, Staphylococcus aureus, Streptococcus faecalis and Yersinia enterocolitica.*

Food and environmental samples

A variety of foods and enviro nmental samples collected by the Canadian Food Inspection Agency staff and submitted for routine microbiological analysis by the Food Laboratory, Laboratory Services Division were examined. The test commodities include: 46 egg products (egg, egg powder, liquid egg mix and egg environmental swabs), 45 fruit and vegetable samples, 111 dairy samples (cheese, ice cream, milk products and dairy environmental swabs) and 364 meat samples (RTE meats and meat environmental swabs). In addition, pure cultures of *Listeria* were spiked into *Listeria*-free samples of milk powder, ice cream, cheese and egg powders.

MICROBIOLOGICAL ANALYSIS

Standard culture technique

All samples were analysed using the standard culture techniques recommended by Health Canada (MFHPB-30, 1994; MFLP-74, 1994). According to the standard culture techniques, sample preparation varies for different products: for foods, add 50g of sample to 450 ml of *Listeria* enrichment broth (LEB, Difco), and for environmental samples, add 25 g or m l to 225 ml LEB. Stomach for thorough mixing and incubate for 48 h at 30 C. At 24 and 48 h, mix the LEB culture and inoculate 0.1 ml into 9.9 ml of modified Fraser broth (mFB) and incubated for 24-48 h at 35 C. After selective enrichment samples were strea ked onto Oxford (OXA, Oxoid) and PALCAM (PAL, BDH) selective agar plates for the isolation of *Listeria*.

Purified *Listeria monocytogenes* isolates were confirmed biochemically and serologically according to the standard culture method. Isolates were also c onfirmed using the Vitek (bioMerieux, Hazelwood, MI, USA) microbiological identification system.

ALOA™ *Listeria* technique

The ALOA™ *Listeria* medium (AES Laboratories, France) contains pre -made selective/ differential *Listeria* ALOA agar plates. Suspect *Listeria* isolates were streaked from the 24h and 48h preenriched half strength Fraser broth cultures, incubated at 30 C, streaked onto the ALOA agar plates, then incubated at 35 -37°C for 24 and 48h. Presumptive positive isolates identified on the indicator p lates were then confirmed as indicated above.

RESULTS AND DISCUSSION

One hundred and fifty three of the 566 pre -enrichment foods and environmental samples tested were found to contain hemolytic *Listeria* species by both the standard culture technique and the ALOA™ *Listeria* plating media (Table 1). These samples consisted of 46 egg products (eggs, liquid egg mix, egg powder and egg environmental swabs), 45 fruit and vegetable samples, 111 dairy products (cheese, ice cream products and dairy environmental swabs), and 364 meat samples (RTE meats and meat environment swabs). The results obtained with the ALOA™ *Listeria* system were in agreement with the standard culture technique in the analysis of naturally contaminated and spiked samples.

The specificity of the ALO A™ medium for *Listeria* was tested using pure various cultures hemolytic and non-hemolytic *Listeria* and non-*Listeria* Gram positive and Gram negative bacteria. The ALOA™ plates were able to pick up all of the *Listeria* strains tested, as evidenced by the appearance of typical *Listeria* colonies on subsequent plating onto the indicator media, Oxford and PALCAM (Table 2). The ALOA plating medium was also able to distinquish between hemolytic and non hemolytic *Listeria* as was evident by the metabolism of a substr ate by phospholipase and the presence of a halo around the blue hemolytic positive *Listeria monocytogenese* and *L. ivanovii* colonies. Differentiation between *L. monocytogenese* and *L. ivanovii* was accomplished by further biochemical testing as indicated in M ethods.

The detectability of the AOLA™ *Listeria* plating media was tested using naturally contaminated samples with low levels of *Listeria monocytogenese , L. ivanovii, and L. innocua* suspended in TSA-YE, enriched overnight in trypticase soya broth at 35 -37C and diluted to approximately1-5 cfu/ ml. The spiked foods were previously determined not to contain Listeria species but did contain other naturally occurring non -*Listeria* isolates. *Listeria* were isolated from all plating media used, Oxford, PALCAM and ALOA. These results suggest that the ALOA plating medium reliably detects low numbers of *Listeria* in enrichment broth cultures, even in the presence of competing non -target bacteria.

Using the ALOA™ *Listeria* plating media allows for the detection of hemolytic *Listeria monocytogenes* and *L. ivanovii* by eliminating the need to test indiscriminately for all *Listeria* species. This then allows for simple biochemical testing to differentiate between *L. monocytogenese* and *L. ivanovii* and enables earlier identification of *L. monocytogenes* with a time savings of 24-48h. The ALOA™ *Listeria* plating technique produced no false negative and no false positive results in the analysis of the foods and environmentals tested.

We conclude that the AES Laboratories ALOA ™ hemolytic *Listeria* technique appears to be a reliable addition to the standard selective plating media, Oxford and PALCAM, for the commodities tested.

ACKNOWLEDGEMENTS

The authors would like to thank Mary Burzynski and the staff of the Food Laboratory, Laboratory Services Division, Ottawa, for their valuable assistance.

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TABLE 1

PERFORMANCE OF THE ALOA ™ HEMOLYTIC LISTERIA PLATING MEDIUM IN THE ANALYSIS OF FOODS AND ENVIRONMENTAL SAMPLES

Product Type	No. Samples Tested	No. Test Results Positive By Both Methods	Standard Culture Technique		ALOA™ Medium	
			No. +ve	No. False -ve	No. +ve	No. False -ve
Fruit and Vegetable Samples	45	7	7	0	7	0
Egg Samples	46	12	12	0	12	0
Dairy Samples	111	10	10	0	10	0
Meat Samples	364	124	124	0	124	0
Total Tested	566	153	153	0	153	0

Enrichment broths of foods and environmental samples were incubated following the standard culture method, then plated on the ALOA ™, Oxford and PALCAM agar plates. Positive, typical hemolytic *Listeria* colonies observed on indicator plate; negative, no typical hemolytic *Listeria* colonies observed on indicator plate. Analysis of these samples was then completed following the standard culture technique as described in Methods.

TABLE 2

PERFORMANCE OF THE ALOA ™ HEMOLYTIC *LISTERIA* PLATING MEDIUM IN THE ANALYSIS OF PURE CULTURES OF *LISTERIA*, NON-HEMOLYTIC *LISTERIA* AND NON-*LISTERIA* BACTERIA

Bacteria	Number Tested	Number Positive		Number Negative	
		Oxford and PALCAM agar plate	ALOA agar plate	Oxford and PALCAM agar plate	ALOA agar plate
non-*Listeria*	20	0	0	20	20
Listeria	18	18	18	0	0

Cultures of hemolytic *Listeria*, non-hemolytic *Listeria* and non-*Listeria* bacteria were suspended at approximately 10^9 cfu/ml and detected by plating on Oxford, PALCAM and ALOA agar. Positive, typical *Listeria* colonies observed on indicator plate; negative, no typical *Listeria* colonies observed on indicator plate.