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Cloth-Based Hybridization Array System for the Identification of Food-Borne *Listeria* and confirmation of *Listeria monocytogenes*

John Z. Xue[1], Franco Pagotto[2], Burton W. Blais[1*]

[1]*Ottawa Laboratory (Carling), Canadian Food Inspection Agency, Ottawa, Ontario, Canada*
[2]*Listeriosis Reference Service, Bureau of Microbial Hazards, Health Canada, Ottawa, Ontario, Canada*

Abstract: A method was developed for the rapid identification of presumptive *Listeria species* colonies isolated from foods by standard culture on agar plates. Key indicator genes for *L. monocytogenes*, *hlyA* and *inlJ*, as well as an rRNA intergenic sequence marker for most members of the genus *Listeria*, are amplified in a multiplex PCR incorporating digoxigenin-11-dUTP, followed by hybridization of the amplicons with an array of specific oligonucleotide probes immobilized on polyester cloth, and subsequent immunoenzymatic assay of the bound digoxigenin label. This system also incorporates a simple internal amplification control (IAC) to gauge PCR inhibition. The cloth-based hybridization array system facilitated the sensitive and specific detection of multiple *L. monocytogenes* gene markers in a single assay procedure. The method exhibited 100% exclusivity and inclusivity characteristics in the analysis of pure cultures of different *L. monocytogenes* strains, other listeriae and several non-*Listeria* bacteria.

Key words: *Listeria monocytogenes*, identification, PCR, probes, array

* Corresponding author. mailing address: Ottawa Laboratory (Carling), Canadian Food Inspection Agency, Bldg. 22, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6, Canada, Tel: 001-613-759-1267, Fax: 001-613-759-1260, E-mail: burton.blais@inspection.gc.ca

Introduction

Outbreaks of listeriosis caused by *Listeria monocytogenes*, are relatively rare compared to other food-borne diseases; however, the mortality rate for infected individuals is approximately 25%, underscoring the importance of this pathogen as a causative agent of food-borne illness (Vazquez-Boland et al. 2001). Immunocompromised individuals and pregnant women are the most at risk. Therefore, widespread contamination of *L. monocytogenes* in food manufacturing environments and related foods is a growing concern as currently no control measures can entirely eliminate the risk associated with this food-borne pathogenic bacterium (Chasseignaux et al. 2001; Peccio et al. 2003; Thévenot et al. 2006). Risk of human illness varies with food products and ready-to-eat (RTE) meats that are consumed without further processing are the greatest concern (Pradhan et al. 2009; Ingham et al. 2010). These RTE meat products may become contaminated post manufacturing and the pathogen may grow to high numbers on the finished product before being consumed.

L. monocytogenes is one of six species of gram positive bacteria in the genus *Listeria*, and the only species associated with food-borne human illness (Vazquez-Boland et al. 2001). *L. monocytogenes* are motile, extremely hardy (resistant to desiccation as well as high and low temperatures), psychrotrophic and facultatively anaerobic. Thirteen different serotypes of *L. monocytogenes* have been characterized, based on somatic (O) and flagellar (H) antigens (Graves et al. 2007). Of these, serotypes 1/2a, 1/2b, and 4b account for over 95% of all cases of listeriosis, with serotype 4b being most commonly implicated in outbreaks (Graves et al., 2007; Gorski, 2008). The utility of various genetic markers detectable by polymerase chain reaction (PCR) techniques has been demonstrated for the identification and characterization of *L. monocytogenes*. One of the most widely recognized gene markers for *L. monocytogenes* is *hlyA*, encoding a hemolysin involved in the pathogenesis of this organism (Mengaud et al. 1988). Other genes proposed as markers for the identification of virulent *L. monocytogenes* strains include: *gtcA*, which is

involved in cell wall teichoic acid glycosylation of serotype 4 strains (Promadej et al. 1999); *lmo*, a gene encoding a putative peptidoglycan-bound protein associated with serotype 1/2a and 1/2c strains (Zhang and Knabel 2005); and *inlJ* (Liu et al. 2007), encoding an internalin (LPXTG protein) allowing the pathogen to invade mammalian cells (Sabet et al. 2005). Recent attempts in our laboratory to utilize some of these markers for determination of the virulence potential of isolates revealed that PCR-amplified *inlJ* sequences were present in a small panel of different *L. monocytogenes* strains representing a variety of serotypes, suggesting the potential applicability of this gene as a species-specific marker (Blais, 2010, unpublished data).

One approach for the identification of *L. monocytogenes* isolated from foods by standard culture techniques utilizes PCR amplification of *hlyA* gene sequences with gel electrophoresis analysis of the amplicon (Blais et al. 2002). This simple method enables confirmation of presumptive *Listeria species* isolated on plating media within a much shorter timeframe than afforded by traditional biochemical techniques. Limitations of this approach are the possible occurrence of *L. innocua* strains bearing *hlyA* (Johnson et al. 2004), and the fact that the assessment of results is based on mere visualization of a DNA fragment of a given size, without provisions for confirmation of amplicon identity or inherent quality control measures for the PCR. Multiplex PCR techniques in which several gene sequences are co-amplified in a single reaction are advantageous in that they allow the interrogation of isolates for several markers which can aid in their definitive identification. The identification of specific amplicons generated by multiplex PCR using a standard thermal cycler can be achieved by hybridization with an array of amplicon-specific DNA probes. However, to ensure the universal availability of such an approach to food microbiology testing laboratories, the hybridization technique must be simple to operate and utilize readily available materials and reagents.

We have previously described a simple low density DNA array technology, termed cloth-based hybridization array system (CHAS), in which strips of macroporous polyester cloth bearing immobilized arrays of capture probes are used in a rapid reverse dot blot hybridization procedure for detection of multiplex PCR products. The use of polyester cloth as a DNA adsorbent for nucleic acid hybridization assays is advantageous in that it provides a cost-effective support yielding improved reaction kinetics (i.e., short reaction times) due to a large and readily accessible surface, and the ease with which the support can be washed between reaction steps to remove unbound reagents. Approaches have been developed for a variety of food microbiology applications, including the determination of various marker genes associated with *Salmonella enterica* subsp. *enterica* serotype Typhimurium DT104 (Gauthier and Blais, 2004), and the identification of *Escherichia coli* O157:H7 isolates (Martinez-Perez and Blais, 2010).

We describe here the adaptation of the CHAS technique for the identification of presumptive *Listeria species* isolates from foods based on the amplification of *hlyA* and *inlJ* marker gene sequences associated with this pathogen. This CHAS method includes a simple internal amplification control (IAC) system for PCR inhibitors based on the incorporation of a primer pair with complementary 3' ends (Leggate and Blais, 2006), as well as an additional quality control element involving amplification of the intergenic region of the 16S-23S rRNA operon, IGS, highly conserved among most members of the genus *Listeria* (Graham et al. 1997).

Materials and Methods

Bacterial strains and culture conditions. A total of 75 different non-*Listeria*, *Listeria* spp. and *L. monocytogenes* strains were selected from the Ottawa Laboratory Carling (OLC) culture collection of the Canadian Food Inspection Agency (Tables 1, 2 and 3). All non-*Listeria* bacteria were grown on trypticase soy agar (TSA) at 37 ℃ for 24 h. All *Listeria* bacteria were grown on brain heart infusion (BHI) agar at 30 ℃ for 24 h.

Preparation of bacterial lysates. Bacterial lysates were prepared by picking a single colony isolated on plating medium and suspending in 100 µL of 1% (v/v) Triton X-100 (Sigma Aldrich Co., St. Louis, MO, USA), followed by heating at 80 ℃ for 5 min.

***Listeria monocytogenes* multiplex PCR.** Sequences for oligonucleotide primers (Sigma-Genosys, Oakville, ON, Canada) targeting *hlyA*, *inlJ* and IGS, as well as internal amplification control (IAC) primers, are described in Table 4. For PCR, 5 µL of bacterial lysate was added to 45 µL of reaction mixture containing 2.5 units HotStar Taq and 1 × HotStar PCR buffer (Qiagen Inc., Mississauga, ON, Canada), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 10 µM alkali-stable digoxigenin (DIG)-11-deoxyuridine triphosphate (dUTP) (Roche Diagnostics, Laval, QC, Canada), 25 µg non-acetylated bovine serum albumin (B8667, Sigma Aldrich Co.), 0.2 µM of each *hlyA* and *inlJ* primer, 0.4 µM of each IGS primer and 0.1 µM of each IAC primer. The PCR was carried out in a Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany) using the following conditions: initial denaturation cycle at 94 ℃ for 15 min, followed by 35 cycles of denaturation at 94 ℃ for 30 s, primer annealing at 50 ℃ for 30 s, and primer extension at 72 ℃ for 1 min 30 s, with an additional extension at 72 ℃ for 2 min following the last cycle.

Table 1. Performance of the Lm CHAS in the analysis of various non-*Listeria* and *Listeria* spp. bacteria[a]

OLC no.	Description	Lm CHAS result[b]			
		hlyA	*inlJ*	IGS	IAC
23	*Escherichia coli* ATCC 11775	-	-	-	+
32	*Pseudomonas aeruginosa* ATCC 10145	-	-	-	+
59	*Salmonella typhimurium* ATCC 14028	-	-	-	+
161	*Bacillus subtilis* ATCC 6051	-	-	-	+
646	*Staphylococcus epidermidis* ATCC 14990	-	-	-	+
4	*Listeria innocua* ATCC 33090	-	-	+	+
9	*Listeria innocua*	-	-	+	+
267	*Listeria innocua*	-	-	+	+
5	*Listeria ivanovii* ATCC 19119	-	-	+	+
10	*Listeria ivanovii*	-	-	+	+
287	*Listeria ivanovii*	-	-	+	+
249	*Listeria seeligeri*	-	-	+	+
11	*Listeria seeligeri*	-	-	+	+
544	*Listeria welshimeri*	-	-	+	+
12	*Listeria welshimeri*	-	-	+	+
13	*Listeria grayi*	-	-	-	+

[a]An isolated colony from a pure culture of each strain grown on TSA was lysed and then subjected to the Lm CHAS procedure as described in Methods. OLC, Ottawa Laboratory Carling culture collection; ATCC, American Type Culture Collection.

[b]Reactivity in the CHAS for each amplicon-specific probe feature: +, blue spot; -, no spot.

***Listeria monocytogenes* CHAS (Lm CHAS) procedure.** The oligonucleotide probes (Sigma-Genosys) specific for the *hlyA*, *inlJ*, IGS and IAC amplicons are described in Table 5. Polyester cloth (DuPont Sontara 8100) strips (2 × 6 cm), bearing a printed sample location grid with 5 cells, were manufactured by contract with Apace Screen Printing & Enterprises Ltd (Ottawa, Ontario, Canada). These were washed with 95% (vol/vol) ethanol, followed by rinsing with deionized distilled water on a filter with vacuum suction. The strips were placed in an uncovered petri dish and air dried, stored in a drawer or cupboard, overnight at room temperature. The oligonucleotide probes were separately diluted to 14.6 µM in high salt buffer (HSB) (0.1 M Tris/HCl [pH 8], 0.01 M $MgCl_2$ and 0.15 M NaCl), then heated for 10 min at 100 °C (to disrupt any possible secondary structures), and snap chilled on ice. The probe solutions were diluted with a sufficient volume of 95% (v/v) ethanol to give final coating solutions containing 10 µM of probe in HSB containing 30% (v/v) ethanol.

Probes (7 µl) were pipetted in discrete spots on the cloth strip, followed by incubation in an uncovered petri dish at 37 °C for 30 min. Probes were cross-linked to the cloth by exposing the strips to UV light (254 nm, 120 mJ/cm^2) using a Stratalinker® UVcross-linker (Stratagene, La Jolla, CA, USA). The strips were blocked by incubating for 1 h at 37 °C with 1 ml hybridization solution (5 × SSC [1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.02% [w/v] SDS, 0.1% [w/v] N-lauroyl sarcosine and 1% [w/v] protein blocking reagent [Bio-Rad, Hercules, CA, USA]), after which they were washed five times by saturating strips with 0.01 M phosphate-buffered saline (pH 7.2, 0.15 M NaCl) (PBS) containing 0.05% (v/v) Tween 20 (PBST) on a filter under vacuum suction. The resulting probe-coated cloth strips were air-dried and stored in a closed container at 4 °C until use. The probe-coated cloth strips were stable for at least 4 months when stored dry at 4 °C.

For the assay of DIG-labelled amplicons, 40 µl of multiplex PCR product was denatured by heating at 100 °C for 10 min, snap chilled on ice, and mixed with 960 µl of ice-cold hybridization solution containing 50% (v/v) formamide. The entire mixture (1 ml) was pipetted onto a strip of probe-coated cloth and incubated for 10 min at 45 °C, followed by washing with PBST on a filter under vacuum suction. All subsequent incubations were carried out at room temperature. Strips were saturated with 1 ml of anti-digoxigenin Fab fragments-peroxidase conjugate (Roche) diluted 1:2000 in PBST containing 0.5% (w/v) protein blocking reagent (Bio-Rad) and incubated for 10 min. After washing with PBST, the strips were saturated with 1 ml tetramethylbenzidine (TMB) membrane peroxidase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA), and incubated for 10 min. Reactions were graded qualitatively as follows: positive (blue spot), negative (no spot).

Table 2. Performance of the Lm CHAS in the analysis of serotyped *L. monocytogenes* strains[a]

		Lm CHAS result[b]			
OLC no.	Description	*hlyA*	*inlJ*	IGS	IAC
2	*L. monocytogenes* ATCC 15313 (serotype 1/2a)	+	+	+	+
523	*L. monocytogenes* serotype 1/2c	+	+	+	+
524	*L. monocytogenes* serotype 1/2c	+	+	+	+
525	*L. monocytogenes* serotype 4c	+	+	+	+
526	*L. monocytogenes* serotype 4e	+	+	+	+
527	*L. monocytogenes* serotype 4d	+	+	+	+
538[c]	*L. monocytogenes* serotype 4bx	+	+	+	+
521	*L. monocytogenes* serotype 4b	+	+	+	+
529	*L. monocytogenes* serotype 3a	+	+	+	+
530	*L. monocytogenes* serotype 3b	+	+	+	+
531	*L. monocytogenes* serotype 3c	+	+	+	+
532	*L. monocytogenes* serotype 7	+	+	+	+
533	*L. monocytogenes* serotype 1/2b	+	+	+	+
535	*L. monocytogenes* serotype 1/2a	+	+	+	+
846	*L. monocytogenes* serotype 4c	+	+	+	+
847	*L. monocytogenes* serotype 4c	+	+	+	+
848	*L. monocytogenes* serotype 4c	+	+	+	+
849	*L. monocytogenes* serotype 4c	+	+	+	+
850	*L. monocytogenes* serotype 4c	+	+	+	+
942	*L. monocytogenes* serotype 4c	+	+	+	+
943	*L. monocytogenes* serotype 4c	+	+	+	+
944	*L. monocytogenes* serotype 4c	+	+	+	+
949	*L. monocytogenes* serotype 1/2a	+	+	+	+
950	*L. monocytogenes* serotype 1/2a	+	+	+	+
951	*L. monocytogenes* serotype 1/2a	+	+	+	+
952	*L. monocytogenes* serotype 1/2a	+	+	+	+
953	*L. monocytogenes* serotype 4b	+	+	+	+
954	*L. monocytogenes* serotype 4b	+	+	+	+
955	*L. monocytogenes* serotype 4b	+	+	+	+
956	*L. monocytogenes* serotype 4b	+	+	+	+
964	*L. monocytogenes* serotype 4a	+	+	+	+
965	*L. monocytogenes* serotype 4a	+	+	+	+
966	*L. monocytogenes* serotype 4a	+	+	+	+
967	*L. monocytogenes* serotype 4a	+	+	+	+
968	*L. monocytogenes* serotype 4a	+	+	+	+

[a]An isolated colony from a pure culture of each strain grown on TSA was lysed and then subjected to the Lm CHAS procedure as described in Methods. With the exception of one strain obtained from the American Type Culture Collection (ATCC), all of the serotyped *L. monocytogenes* strains were originally obtained from F. Pagotto and J. Farber, Listeriosis Reference Service for Canada (Bureau of Microbial Hazards, Health Canada) and deposited in the Ottawa Laboratory Carling (OLC) culture collection.

[b]Reactivity in the CHAS for each amplicon-specific probe feature: +, blue spot; -, no spot.

[c]Implicated in a listeriosis outbreak in the UK between 1987 and 1989, traced to contaminated pâté (McLauglin et al. 1991).

Results and Discussion

The intended application of the Lm CHAS is the confirmation of presumptive *L. monocytogenes* isolated on plating media using standard culture techniques. The method has its basis in the amplification of the putative *L. monocytogenes* marker gene sequences *hlyA* and *InlJ*, as well as a *Listeria* genus marker, IGS, and an internal amplification control element (IAC), followed by hybridization of the amplicons with an array of oligonucleotide probes immobilized on a strip of polyester cloth (with immunoenzymatic detection of the captured amplicons incorporating a digoxigenin label). Typical results obtained in the assay of lysed *L. monocytogenes* colonies are demonstrated in Figure 1, which clearly shows the pattern of positive reactions

obtained on the array as a result of hybridization of the different amplified DNA sequences with their respective immobilized probes. To ascertain the reliability of this technique as a tool for the characterization of colonies, it was deemed necessary to determine (1) the range of the quantity of material (i.e., number of *L. monocytogenes* cells, or cfu) eliciting a positive reaction for each marker, (2) the exclusivity and inclusivity characteristics of the assay using a variety of different target and non-target bacterial strains, and (3) the ability of the assay to detect the marker genes in target bacteria grown on a variety of plating media commonly utilized in the recovery of *L. monocytogenes* from foods.

A suspension of *L. monocytogenes* (ATCC 15313) cells was serially diluted 10-fold in lysis solution and each dilution was subjected to the Lm CHAS procedure. A positive reaction was obtained with each probe feature in the array using ca. 10^2-10^6 cfu per PCR reaction (not shown). This suggests that the Lm CHAS exhibited suitable detectability characteristics providing for the reliable assay of a typical colony isolated on plating media (we have determined that one colony grown on BHI agar contains approximately 10^8 cfu). The broad range of cell densities (at least four orders of magnitude) over which positive results were obtained demonstrates the robustness of the method with respect to analyst-specific variations in technique.

The exclusivity characteristics of the Lm CHAS were examined using single colony lysates from pure cultures of a total of 16 non-*L. monocytogenes* strains, including 11 listeriae and 5 non-*Listeria* bacteria. None of the non-*Listeria* bacteria tested produced positive reactions for any of the gene marker features, with only the IAC probe eliciting a positive reaction, indicating that the amplification, hybridization and immunoenzymatic

Table 3. Performance of the Lm CHAS in the analysis of *L. monocytogenes* strains isolated from foods and their manufacturing environments[a]

OLC no.	Description	Source/information	Lm CHAs result[b]			
			hlyA	*inlJ*	IGS	IAC
6	*Listeria monocytogenes*	Scott A	+	+	+	+
17	*L. monocytogenes*	Egg plant environmental	+	+	+	+
19	*L. monocytogenes*	Cheese plant environmental	+	+	+	+
65	*L. monocytogenes*	Meat plant environmental	+	+	+	+
66	*L. monocytogenes*	Meat plant environmental	+	+	+	+
67	*L. monocytogenes*	Meat plant environmental	+	+	+	+
68	*L. monocytogenes*	Pizza, Italian sausage	+	+	+	+
69	*L. monocytogenes*	Chicken parmigiana	+	+	+	+
70	*L. monocytogenes*	Chicken, bacon, processed cheese	+	+	+	+
71	*L. monocytogenes*	Veal, bacon, processed cheese	+	+	+	+
75	*L. monocytogenes*	Meat plant environmental	+	+	+	+
78	*L. monocytogenes*	Pork spareribs	+	+	+	+
79	*L. monocytogenes*	Meat plant environmental	+	+	+	+
81	*L. monocytogenes*	Polish sausage	+	+	+	+
82	*L. monocytogenes*	Cheese plant environmental	+	+	+	+
84	*L. monocytogenes*	Ice cream plant environmental	+	+	+	+
85	*L. monocytogenes*	Dairy plant environmental	+	+	+	+
86	*L. monocytogenes*	Dairy plant environmental	+	+	+	+
99	*L. monocytogenes*	Pouligny cheese, raw milk	+	+	+	+
179	*L. monocytogenes*	Ground beef	+	+	+	+
211	*L. monocytogenes*	Cheese	+	+	+	+
223	*L. monocytogenes*	Cheese	+	+	+	+
230	*L. monocytogenes*	Raw milk cheese	+	+	+	+
925	*L. monocytogenes*	Roast beef	+	+	+	+

[a]An isolated colony from a pure culture of each strain grown on TSA was lysed and then subjected to the Lm CHAS procedure as described in Methods. With the exception of one strain obtained from the American Type Culture Collection (ATCC), all strains were drawn from the Ottawa Laboratory Carling (OLC) culture collection and were originally isolated from a variety of foods and their manufacturing environments by Canadian Government food inspection staff during the previous 20 year period.

[b]Reactivity in the CHAS for each amplicon-specific probe feature: +, blue spot; -, no spot.

Table 4. Oligonucleotide primer sequences used in the two newly devised *Listeria monocytogenes* multiplex PCR-cloth-based hybridization array system procedures

Primer	Sequence (5' → 3')	Amplicon size (bp)	Reference
IGS-F	CTA TAG CTC AGC TGG TTA GAG	275	Graham et al. (1996)
IGS-R	TTC TCG GTT ACT TGT GTC A		
hlyA-F	GAA CCT ACA AGA CCT TCC AG	193	Designed for this study from *hlyA* gene sequence in Mengaud et al. (1988)
hlyA-R	TTT CCG CTT ACG GCA GCA TC		
inlJ-F	TGT AAC CCC GCT TAC ACA GTT	238	Liu et al. (2007)
inlJ-R	AGC GGC TTG GCA GTC TAA TA		
IAC-1	CAT AAT ATC ACT CGC GTC CGT TGA AGC TTA	40	Leggate and Blais (2006)
IAC-2	GAC GAA ATC GTA AGC TTC AA		

detection phases of the assay were functioning correctly (Table 1). With the exception of *L. grayi*, a non-pathogenic *Listeria*, all of the other *Listeria* spp. tested gave a positive result with the IGS feature, as well as the IAC, but not for any of the *L. monocytogenes*-specific markers. Thus, the Lm CHAS exhibited 100% exclusivity with respect to the *L. monocytogenes* markers.

The reactivity of the different markers in the Lm CHAS was examined using a panel of *L. monocytogenes* representing a variety of strains with defined serotypes from the Listeriosis Reference Service of Canada (Table 2), as well as a number of isolates recovered from various foods and related commodities sampled by CFIA inspection staff during the preceding 20 year period (Table 3). All strains produced positive reactions with the species-specific markers *hlyA* and *inlJ*, as well as the genus marker IGS, thus demonstrating 100% inclusivity with respect to the identification of *L. monocytogenes* strains from different sources. Despite previous observations regarding the utility of *inlJ* detection in virulence assessment of *L. monocytogenes* (Liu et al. 2007), the results of this study show that the *inlJ* sequences targeted by the PCR were present in all *L. monocytogenes* strains tested regardless of virulence potential as might be inferred by serotype (Table 2). For instance, while serotype 4a isolates are generally not associated with human illness, all 5 different *L. monocytogenes* strains of this serotype examined in the present study were found to bear the *inlJ* marker. Notwithstanding, the possibility that serotypes infrequently associated with food-borne illness may be virulent cannot be discounted.

The recovery of *L. monocytogenes* from foods by standard culture techniques involves the use of several different types of plating media from which presumptive colonies are picked for further characterization. In the official Canadian method, a variety of specialized plating media are used for the selective isolation and characterization of *L. monocytogenes*, including Oxford agar, Palcam agar, chromogenic media, and blood agar (TSA containing horse blood) (Pagotto et al. 2001). Some plating media may contain agents that can

Table 5. Oligonucleotide probe sequences used in the newly devised *Listeria monocytogenes* multiplex PCR-CHAS procedures

Oligonucleotide probe	Sequence (5' → 3')	Reference
hlyA probe	ATC CTC CTG CAT ATA TCT CAA GTG TGG CGT ATG GCC GTC AAG TTT ATT TG	Designed for this study from Mengaud et al. (1988)
IGS probe	GCA CGC CTG ATA AGC GTG AGG TCG ATG GTT CGA GTC CAT TTA GGC CCA CT	Designed for this study from sequence in Graham et al. (1997)
inlJ probe	GAC CTA ACA CAC AAC ACA CAA TTA ATA TAT TTT CAA GCT GAA GGA TGT AG	Designed for this study from NCBI accession number FM242711
IAC probe	CAT AAT ATC ACT CGC GTC CGT TGA AGC TTA CGA TTT CGT C	Leggate and Blais (2006)

be absorbed by surface colonies and ultimately interfere with the PCR method. To demonstrate the applicability of the Lm CHAS in the identification of *L. monocytogenes* grown on different plating media, a pure culture (ATCC 15313) was streaked onto a variety of different media commonly used in the isolation and characterization of food-borne isolates, and isolated colonies grown thereon were subjected to the Lm CHAS procedure. Colonies of *L. monocytogenes* sampled from all of the selective and differential plating media tested produced strong reactions for each feature in the array, the intensity of which was similar to the reactions obtained with colonies grown on BHI agar (Figure 1). Therefore, the performance of the Lm CHAS was not affected by the composition of the agar medium upon which test colonies were grown, and the method may be deemed suitable for use in the assay of presumptive colonies isolated on plating media commonly used in the recovery of this pathogen from foods.

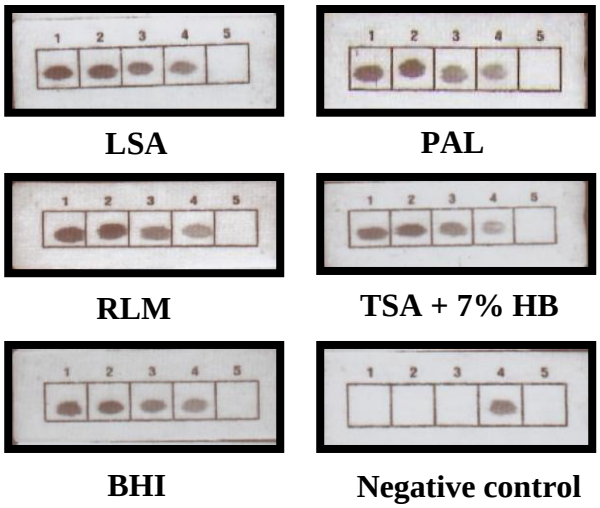

Figure 1. Analysis of *L. monocytogenes* grown on different plating media by the Lm CHAS. Isolated colonies of *L. monocytogenes* grown on different plating media (LSA, Listeria selective agar; PAL, Palcam agar; RLM, RAPID' L.mono chromogenic agar; TSA + 7% HB, tryptic soy agar with defibrinated horse blood; BHI, brain heart infusion agar; Negative Control, lysis solution devoid of bacteria), then lysed and subjected to the Lm CHAS procedure as described in Methods. Probes were arrayed as follows: 1, *hlyA*; 2, *inlJ*; 3, IGS; 4, IAC; 5, empty (not used).

We have demonstrated the utility of the Lm CHAS method as a tool for the definitive identification of *L. monocytogenes* colonies isolated on plating media. This method has several advantages over standard biochemical tests used for colony identification, namely, the ability to provide same day results without the need for further purification of colonies (thus significantly reducing the turnaround time for the provision of results to inform regulatory actions), and the flexibility to assay presumptive colonies at different junctures during the enrichment culture process (i.e., identification of *L. monocytogenes* directly on selective agars, hemolysis plates and non-selective media). Furthermore, the use of a colony allows for downstream analyses such as epidemiological investigations using, for example, pulsed-field gel electrophoresis, which can be readily accomplished in the context of the present approach since only a portion of the colony is required for the CHAS procedure. The use of two species-specific markers, *inlJ* and *hlyA*, and the inclusion of two quality control features (IGS detection as a control for DNA extraction, amplification and detection, and IAC as a control for PCR inhibition) enhance the reliability of the method as a confirmatory tool. In this assay the genes acting as the basis for the species-specific markers are key elements in the pathogenic mechanism of *L. monocytogenes*, and their discernment in food-borne isolates will contribute significantly to risk-based regulatory decision making.

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