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Review

New chromogenic plating media for detection and enumeration of pathogenic *Listeria* spp.—an overview☆

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Abstract

In recent years a number of selective chromogenic plating media for pathogenic *Listeria* spp. have been developed and marketed. Their advantages are direct detection and enumeration of pathogenic *Listeria* spp. utilizing cleavage of substrates by the virulence factor phosphatidylinositol-phospholipase C (PI-PLC) and, to a lesser extent, by phosphatidylcholin-phospholipase C (PC-PLC). There are two groups of such media: the first utilizes cleavage by PI-PLC of L-α-phosphatidyl-inositol, forming a white precipitation zone around the colony, combined with the chromogenic substrate 5-bromo-4-chloro-3-indoxyl-β-D-glucopyranoside for detection of β-D-glucosidase, which occurs in all *Listeria* spp. All *Listeria* spp. produce turquoise colonies on these media which include ALOA™, CHROMagar™ Listeria , BBL™ CHROMagar™ Listeria, and OCLA. The second group of media utilizes 5-bromo-4-chloro-3-indoxyl-myoinositol-1-phosphate, forming blue-turquoise colonies of pathogenic *Listeria* spp. and white colonies of non-pathogenic *Listeria* spp.. BCM™ *Listeria monocytogenes* plating medium, Rapid'L.mono and LIMONO-Ident-Agar belong to this group. Selective chromogenic *L. monocytogenes* plating media offer the attraction of rapid economic detection and enumeration of pathogenic *Listeria* spp. within 24 or 48 h of incubation at 36 ± 1 °C. This overview summarises the characteristics of these chromogenic plating media, reviews important evaluations, and focuses on replacement of conventional by these chromogenic plating media, particularly for applications in the food industry.

Keywords: Pathogenic *Listeria* spp.; Selective chromogenic plating media

1. Introduction

Among the six species of the genus *Listeria* only two are pathogenic, *Listeria monocytogenes* and *L. ivanovii*. *L. monocytogenes* is a rare cause of food-borne disease with fewer than four cases per million individuals per year reported in developed countries (Buchanan and Lindquist, 2000). Fatality rates are 20–30% among hospitalised patients.

L. ivanovii is pathogenic for animals, especially ruminants. Hitchins (2002) compiled reports of seven cases of human listeriosis caused by *L. ivanovii*, at extremely low frequency. *L. ivanovii* is rarely found in foods (e.g., Zschaler, personal community), but its occurrence and contribution to human listeriosis may be underestimated.

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L. monocytogenes has been associated with foods such as raw milk, cheeses (particularly soft-surface-ripened varieties), ice cream, raw vegetables, raw and cooked poultry, raw meats and raw and smoked fish. Large outbreaks of human listeriosis have been traced to soft cheese, raw cabbage, liver paté, pork tongue in aspic (see e.g. Curtis and Lee, 1995). Sporadic or single unrelated cases of human listeriosis are also common in many countries.

The food industry remains concerned about the presence of *L. monocytogenes* in refrigerated, minimally processed, ready-to-eat foods and where modified atmosphere packaging (MAP) is used to extend the shelf life. Preventive systems, such as HACCP, are considered effective for preventing listeriosis outbreaks (Baird-Parker, 1994). Particular problems with *L. monocytogenes* are its ability to survive at low pH (acid tolerance) and high NaCl content (osmotolerance) and other stress conditions (Faleiro et al., 2003; King et al., 2003) as well as its ability to multiply at low temperature.

There are various regulations concerning the microbiological level of *L. monocytogenes* in foods. Tolerance levels in ready-to-eat foods have been introduced in many countries. These depend, to some extent, on the type of food e.g. whether the food supports growth of listeriae, whether it is raw or processed or ready-to-eat, and the projected shelf life. The USA require absence of *L. monocytogenes* in 25 g of foods (“zero tolerance”) while some European countries have a tolerance level of 100 *L. monocytogenes*/g at the point of consumption (European Commission, 1999; DGHM, 2003). The latest draft of the EU regulation on microbiological criteria for foodstuffs recommends for “ready-to-eat food intended for infants and for special medical purposes” no *L. monocytogenes* should be allowed (Anonymous, 2003a).

Rapid detection and enumeration of pathogenic *Listeria* spp. in foods is laborious and time-consuming using conventional culture media. The recovery of low numbers of *L. monocytogenes* from foods and environmental samples requires the use of enrichment cultures followed by selective plating and, where injured organisms are likely to be present, a pre-enrichment step. An overview of conventional culture media and methods is given by Beumer and Curtis (2003) and Loessner et al. (1988). Currently, PALCAM, Oxford and LPM are the most frequently used plating media. They are recommended by the US Food and Drug Administration (FDA), the US Department of Agriculture (USDA), in the ISO detection and enumeration methods (Anonymous, 1997, 1998). National recommendations, e.g. DIN or § 35 LMBG in Germany are based on the ISO standards. *Listeria* spp. growing on these media are detected by the activity of the enzyme β-D-glucosidase, which occurs in both pathogenic and non-pathogenic species. This enzyme (esculinase) cleaves esculin, resulting in greyish-green colonies, and by reaction of the breakdown product esculetin with ferric iron, gives brown-black halos with colonies of all *Listeria* spp. Isolation and confirmation of pathogenic *Listeria* spp. growing in mixed culture with non-pathogenic *Listeria* spp. on such plating media is time-consuming and expensive.

Alternative methods based on nucleic acid, fluorescent antibody or immuno-chromatography techniques need additional equipment and expensive devices as well as enrichment steps (Fitter et al., 1992; Hoffman and Wiedmann, 2001; Becker and Märtlbauer, 2002; Istafanos et al., 2002; Bhagwhat, 2003; Choi and Hong, 2003; Hitchins, 2003).

Chromogenic culture media detecting virulence factors in the target pathogenic *Listeria* spp. are an attractive alternative and should ideally combine rapid detection and enumeration of pathogenic *Listeria* spp. with low cost.

Several virulence genes have been identified in pathogenic *Listeria* spp. Among them, the *hly*A gene encodes a haemolysin, called listeriolysin O that is essential for these strains for invasion of the host cells, lysis of their phagosomes, and subsequent spread. This gene is flanked by the *plc*A-*prf*A operon and the lecithinase operon. The lecithinase operon contains at least two genes involved in virulence: *act*A, required for actin assembly and *plc*B. *plc*B encodes a commonly called lecithinase (phosphatidylcholin-phospholipase C, PC-PLC) involved in cell-to-cell spread. Upstream from *hly*, *plc*A encodes a phosphatidylinositol-specific phospholipase C (PI-PLC) which may contribute to the lysis of the phagosomal membrane. These three virulence genes (*hly, plc*A, *plc*B) occur in the pathogenic *L. monocytogenes* and *L. ivanovii* and in *L. seeligeri* but not in the non-pathogenic *L. innocua, L. welshimeri or L. grayi*. *L. seeligeri* has no PI-PLC or PC-PLC and expresses haemolysin at a very low level. So it appears that this species is non-pathogenic because of the

down-regulation of most of its virulence genes. *L. ivanovii* produces another phospholipase C that is specific for sphingomyelin. This sphingomyelase contributes to the haemolytic activity of *L. ivanovii*. The infective behaviour of *L. ivanovii* is quite similar to that of *L. monocytogenes*. Indeed, this species escapes from the host cell vacuole, induces actin assembly and spreads from cell to cell (Camilli et al., 1991; Gouin et al., 1994; Vàzquez-Boland et al., 2001).

2. Utilization of virulence gene products for developing new plating media

From the virulence gene cluster consisting of *hly*, *pcl*A and *pcl*B, the *hly* gene product has been utilized for confirmation of pathogenic *Listeria* spp. with respect to haemolysis detectable on sheep blood agar as well as producing typical CAMP reactions. The non-pathogenic *Listeria* spp., lacking the *hly* gene, are non-haemolytic.

Recently, a selective blood agar plating medium for detection of pathogenic *Listeria* spp. was developed by Johansson (1998) and evaluated by Johansson et al. (2000). This *L. monocytogenes* blood agar (LMBA) showed best overall results when compared to PALCAM, Oxford and LPM. The ISO/TC 34/SC9 group "Agricultural and food products-Microbiology" also evaluated it. Due to batch-to-batch variations in blood quality it was not considered a suitable replacement for conventional culture media.

Notermans et al. (1991) were the first to utilize the *plc*A gene product phosphatidylinositol-phospholipase C (PI-PLC) for detection of *L. monocytogenes* by an

Table 1
Detection systems of *Listeria* spp. plating media[a]

Plating medium	Substrate for detection of		Colony colour	
	β-D-Glucosidase	PI-PLC[b]	non-pathogenic *Listeria* spp.	*L. monocytogenes*/ *L. ivanovii*
PALCAM; - Oxford LPM; - MOX	Esulin	–	grey, with brown-black surround	grey, with brown-black surround
Harlequin™ Listeria (Lab m)	CHE-β-D-glucopyranoside	–	black	black
BCM® *Listeria monocytogenes* (Biosynth)		5-Bromo-4-chloro-3-indoxyl-myo-inositol-1-phosphate (X-IP)	white	blue-turquoise
Rapid'L. mono (Bio-Rad)	–	X-IP	white	*L. monocytogenes*: blue-turquoise *L. ivanovii*: blue-green with a yellow halo
LIMONO-Ident-Agar (Heipha)	–	X-IP	white	blue-turquoise, surrounded by a white precipitate
ALOA (AES, Biolife)	5-Bromo-4-chloro-3-indoxyl-β-D-glucopyranoside	L-α-Phosphatidyl-inositol	blue-turquoise	blue-turquoise, surrounded by a white precipitate
CHROMagar™ Listeria (CHROMagarBBL™)				
CHROMagar™ Listeria (BD)				
OCLA (Oxoid)				

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[a] *Listeria monocytogenes* blood agar (LMBA) uses haemolysis of sheep blood for detection of pathogenic *Listeria* spp.

[b] Phosphatidylinositol-phospholipase C.

overlay technique with L-α-phosphatidyl-inositol as substrate. Cleavage of this substrate by PI-PLC produces water-insoluble fatty acids around the colonies, with a distinct opaque halo-like precipitation zone. Notermans et al. (1991) confirmed 468 *L. monocytogenes* strains to be positive with this test. Ottaviani et al. (1997a,b) combined this detection system with the detection of the β-D-glucosidase activity of *Listeria* spp. using the chromogenic substrate 5-bromo-4-chloro-3-indoxyl-β-D-glucopyranoside (X-β-D-glu). The medium containing both substrates in a nutrient rich selective agar base is now known as "Agar Listeria according to Ottaviani and Agosti (ALOA™)". Media based on similar detection systems have been introduced by other manufacturers (see Table 1).

Another substrate for the detection of PI-PLC is 5-bromo-4-chloro-3-indoxyl-myoinositol-1-phosphate (X-IP). Cleavage of X-IP (see Fig. 1) by pathogenic *Listeria* spp. results in turquoise colonies. Non-pathogenic *Listeria* spp. are clearly distinguishable as white colonies. Facon and Simon (1998) filed a patent for utilizing X-IP while, in the same year, Schabert and Restaino (1998) filed another patent for utilizing the fluorogenic 4-methylumbelliferyl-myoinositol-1-phosphate in selective chromogenic/fluorogenic culture media for detection of PI-PLC producing bacteria. Commercially available media utilising X-IP for detection of PI-PLC are listed in Table 1. Rapid'L.mono plates are red-brown because they contain phenol red as pH indicator. *L. ivanovii* colonies utilise xylose in the medium to give blue-green colonies with a yellow halo. BCM™ *L. monocytogenes* plating medium (Restaino et al., 1999) and LIMONO-Ident-Agar contain no differentiating carbohydrate or pH indicator system, due to difficulties in distinguishing the carbohydrate-metabolising colonies.

Both substrates work well for detection of PI-PLC and the turquoise colonies of pathogenic *Listeria* spp. are easy to enumerate. Utilising L-α-phosphatidyl-inositol, non-pathogenic *Listeria* spp. may grow in the precipitation zone, which can make it difficult to detect colonies of pathogenic *Listeria* spp. as almost the whole plate is covered by the precipitate. Colonies of *L. monocytogenes* and *L. ivanovii* have similar morphology, colour and precipitation zone on chromogenic *L. monocytogenes* plating media. *L. ivanovii* can subsequently be distinguished from *L. monocytogenes* by production of acids from rhamnose and xylose or by any of the alternative confirmation tests. Very rarely, atypical haemolytic listeria isolates may occur. Procedures to confirm identities of such aberrant isolates are given in a BAM guideline (Anonymous, 2003b).

LIMONO-Ident-Agar additionally combines the cleavage of X-IP in forming turquoise colonies of pathogenic *Listeria* spp. with production of a white precipitate surrounding these colonies. This precipitate is formed by use of selected nutrients, well balanced by nutrient analysis (e.g. see Fig. 2) and addition of a

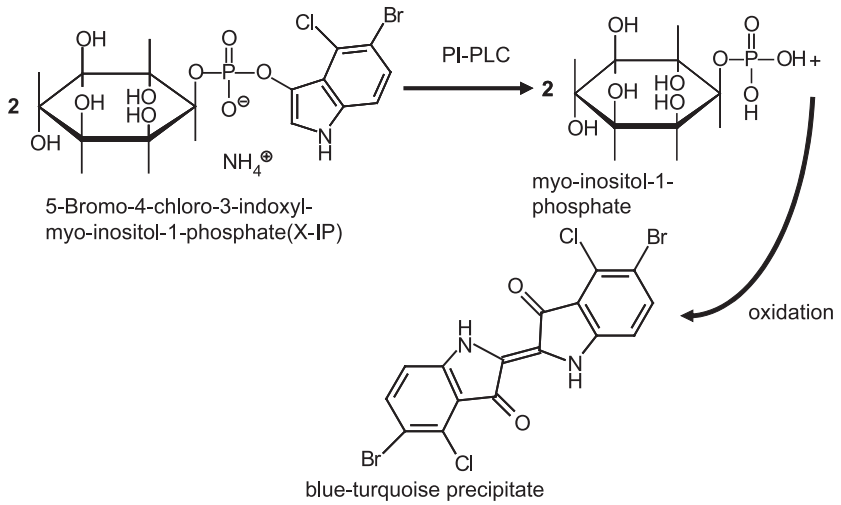

Fig. 1. Detection of phosphatidylinositol-phospholipase C (PI-PLC) by use of X-IP.

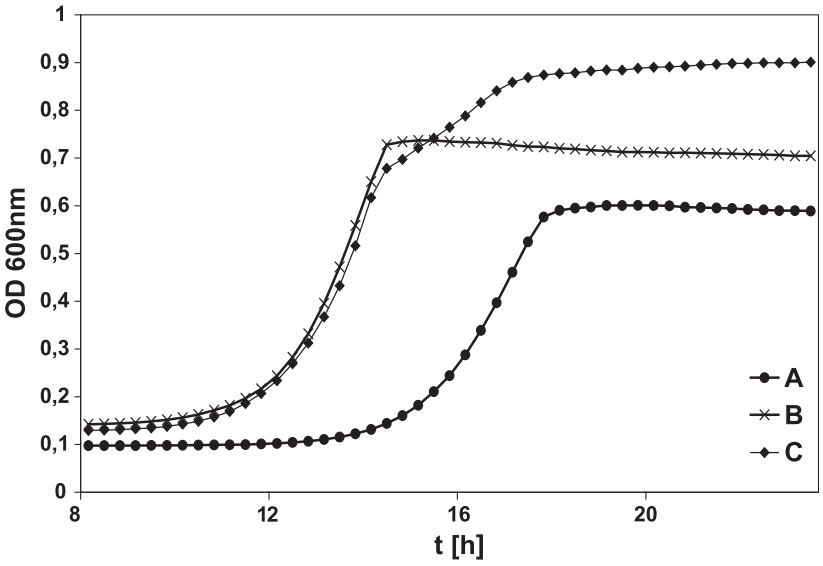

Fig. 2. Bioscreen C comparison of the growth of *L. monocytogenes* using two different peptones (A; B) (pancreatic digests of casein) and the influence of yeast extract (C). Culture densities (OD_{600}) were monitored at 37 °C for 24 h, and each line represents the average data from 10 replicate wells (own unpublished results).

selected phospholipid mixture (Hechelmann et al., 2002; Roczen et al., 2003). Hence, both virulence genes may be involved in forming this zone of precipitation: *plc*A (PI-PLC) and *plc*B (PC-PLC). Recently, Ermolaeva et al. (2003) reported that growing pathogenic *Listeria* spp. in the presence of activated charcoal induced production of PC-PLC. All pathogenic listeria strains tested showed a clear induction of PC-PLC activity measured by a dense halo of precipitation in charcoal-supplemented medium. The precipitation was strongly dependent on the quality of the basic medium (various batches of Brain Heart Infusion).

3. Evaluation of the new chromogenic plating media for pathogenic *Listeria* spp.

The ability of *Listeria* spp. to produce β-D-glucosidase was utilized in the chromogenic Harlequin™ Listeria medium (Smith et al., 2001). *Listeria* spp. cleave cyclohexeno-esculetin-(CHE)-β-D-glucopyranoside and appear as black colonies. The advantage of this medium compared with the conventional esculin-containing media is that the black pigment is retained within the colonies which makes screening for suspect colonies very easy. Smith et al. (1999) described a 34% increase in positive isolations with this medium over Oxford medium. Since no substrates for any of the virulence gene products are incorporated, direct detection of pathogenic *Listeria* spp. is not possible with this medium.

ALOA™ was intensively evaluated by Vlaemynck et al. (2000) with respect to sensitivity, selectivity and electivity in comparison with PALCAM and Oxford, using pure cultures, spiked dairy, egg and meat products. Using ALOA™ they detected 4.3% more positive samples from naturally contaminated dairy and meat products and it was clearly superior to Oxford and PALCAM when samples containing both *L. monocytogenes* and *L. innocua* were examined.

Schuler et al. (2003) obtained similar results using Rapid'L.mono and ALOA™ after 24 and 48 h at 36 ± 1 °C in a rapid *L. monocytogenes* test on smoked salmon. Hechelmann et al. (2001) isolated significantly more *L. monocytogenes* with BCM than with PALCAM from a total of 1633 samples, including raw sausage batters and fresh fermented spreadable sausages (4.8% positve with BCM versus 3.2% positive with PALCAM). No false-positive or false-negative results were detected by confirmation tests (PCR, acids from rhamnose and xylose as well as further confirmation on Rapid'L.mono agar).

Gracieux et al. (2003) evaluated PALCAM, Oxford, Rapid'L.mono and ALOA™ using 15 virulent, 8 hypovirulent and 17 avirulent *L. monocytogenes* strains. Virulence was checked by a plaque-forming assay with human colon adenocarcinoma cell line HT-29 cell monolayers, and by testing the spread of the strains to the liver of mice in vivo. The results showed a positive correlation between the level of virulence and growth on selective plating media. Virulent *L. monocytogenes* grew on all media tested. However, a few hypovirulent and avirulent strains did not grow on PALCAM, Oxford or Rapid'L.mono. Most avirulent strains grew but did not express PI-PLC activity on Rapid'L.mono. ALOA™ detected 92% of the hypo-and avirulent strains. These results indicate that ALOA™ is more sensitive. How far these virulence data are of significance for humans is not clear as yet. Moreover, quality control measures must include hypovirulent (i.e. weak PI-PLC producing) *L. monocytogenes* strains.

Following additional instructions given by the ISO/TC 34/SC 9 group "Agricultural and food products—Microbiology" a ring-trial was performed in 2002 of isolation of *L. monocytogenes* from spiked foods by application of the direct counting method. Normal and weak PI-PLC producers were evaluated. Counts of *L. monocytogenes* and *L. innocua* were similar on ALOA™ and Rapid'L.mono (ready-to-use plates) as well as BCM and LIMONO-Ident-Agar prepared from powder and supplements provided by the manufacturer. Interestingly, numbers of easily-recognised colonies of both *L. monocytogenes* and *L. innocua* significantly increased on ALOA™ and Rapid'L.mono between 24 and 48 h incubation at 36 ± 1 °C to similar levels as seen for BCM and LIMONO-Ident-Agar after 24 h incubation (Table 2). Rapid'L.mono was most selective, LIMONO-Ident-Agar was found to be more sensitive in further experiments. The enrichment method gave similar results using ALOA™, BCM or LIMONO-Ident-Agar (Roczen et al., 2003). The ability to do direct counts of pathogenic *Listeria* spp. is one of the main advantages of using chromogenic *L. monocytogenes* plating media.

Comparing Oxoid Chromogenic Listeria Agar (OCLA, ready-to-use plates) with LIMONO-Ident-Agar, weak PI-PLC producing strains of pathogenic *Listeria* spp. required 40–44 h to produce clearly recognisable precipitation zones around the turquoise colonies, whereas with LIMONO-Ident-Agar, precipitation zones were clearly visible around the turquoise colonies after 24 h of incubation (Reissbrodt, unpublished results).

Various workers have compared results obtained by alternative methods with those using ALOA™, Rapid'L.mono and BCM (Becker and Murphy, 2000; Becker et al., 2001; Hechelmann et al., 2001; Hoffman and Wiedmann, 2001; Istafanos et al., 2002). All these investigations demonstrated the high correlation of results using chromogenic *L. monocytogenes* plating media with results from alternative methods. A comparative validation of chromogenic *L. monocytogenes* plating media by the ISO/TC 34 SC 9 group showed that all the chromogenic plating media tested

Table 2
Enumeration of *L. monocytgenes* and *L. innocua* from spiked foods by direct counting using chromogenic *L. monocytogenes* plating media

Food	Inoculum of *L. mono./* L. innocua (cfu) per 100 ml stomached food suspension	Hours of incubation at 36±1 °C	ALOA	Rapid'L.mono	BCM	LIMONO-Ident-Agar
			counts of *L. mono./ L. innocua*	counts of *L. mono./ L. innocua*	counts of *L. mono./ L. innocua*	counts of *L. mono./ L. innocua*
Three types of cheese from untreated milk	$1.5\times10^4/1.6\times10^4$	24	97/129	112/111	123/102	124/103
Four types of fermented raw sausages	$1.1\times10^4/1.3\times10^4$	24	121/125	111/193	175/184	198/148
Four types of fermented raw sausages	$1.1\times10^4/1.3\times10^4$	48	150/98	169/118	172/195	203/156

0.1 ml of food: saline suspensions (ratio 1:3;1: 6;1:10) plated before spiking, subsequently 0.1 ml of the spiked suspension plated. Counts summarized from nine experiments of each food according to the instructions of the Ring-trial 2002 of the ISO/TC 34/SC 9.

(and LMBA) inhibited competitive organisms more or less equally well. This result was expected since the selectivity of the chromogenic plating media is similar to that of the conventional culture media. Some new combinations of antibiotics (ceftazidime, colistin or polymyxin B, fosfomycin, ofloxacine) were used to inhibit Gram-negative and some Gram-positive bacteria. Nalidixic acid was incorporated to inhibit *Bacillus* spp., particularly the *B. cereus* group which also carries PI-PLC. Cycloheximide or amphotericin B inhibited yeasts and moulds whilst lithium chloride, which is also used in the conventional culture media for *Listeria* spp., improved the selectivity to enterococci. Acriflavin was found unsuitable for chromogenic plating media due to interference with the development of colony colours.

International ring-trials have been performed to compare the chromogenic *L. monocytogenes* plating media with the conventional PALCAM and Oxford media (Anonymous 1995, 1997, 2002). ALOA™, as the first medium on the market, has been more frequently evaluated than others. In the first ring-trials ALOA™ was at least equal to or superior to PALCAM and Oxford with respect to specificity and productivity. In the next evaluations Rapid'L.mono and BCM™ *L. monocytogenes* plating media behaved similarly to ALOA™. Hitchins (2002) found ALOA™ and Rapid'L.mono performed equally well for isolation with respect to the ratio of *L. monocytogenes* to competitors, while BCM and LMBA were judged as moderate in this respect. Rapid'L.mono and ALOA™ have successfully and repeatedly been validated by AFNOR. Karpiskova et al. (2000) proposed the replacement of PALCAM and Oxford media used in the EN ISO 11290-1 method with Rapid'L.mono.

ALOA™ and BCM have been successfully evaluated by the FDA. Eight FDA institutes participated in an evaluation and inter laboratory validation study of BCM compared with PALCAM and Oxford. BCM was superior to these standard plating media with regard to the recovery of *L. monocytogenes* from 2000 food and environmental samples. The published results revealed BCM/Oxford and BCM/PALCAM as the best combinations, superior to Oxford/PALCAM. Testing pure cultures, all of 51 *L. monocytogenes* strains appeared typical on BCM, and 7 of 8 *L. ivanovii* strains gave colonies similar to *L. monocytogenes* (Jinneman et al., 2003).

The BAM protocol (Hitchins, 2003) strongly recommends the use of one of the chromogenic plating media ALOA™, BCM™ *L. monocytogenes*, Rapid'L.mono, or CHROMagar™ Listeria, streaking at 48 h (optionally also at 24 h) from growth on one of the conventional esculin-containing selective isolation agars (Oxford, PALCAM, MOX or LPM). This reduces the problem of masking of *L. monocytogenes*/*L. ivanovii* by *L. innocua* and other non-pathogenic *Listeria* spp.

ALOA™ was preferred by the ISO/TC 34 SC 9 group because its formulation is public. However, in-house preparation of ALOA™ or other chromogenic plating media from basic ingredients according to the given formulas is difficult since performance depends on the correct balance of nutrients (in particular of peptones and yeast extracts, see Fig. 2) and of the best mixture of phospholipids both of which are difficult to standardise in-house (see also report of ISO/TC 34 / SC 9, 2002). Microbiological laboratories should, however, carefully control ready-to-use media and media produced by the laboratories from dehydrates and supplements. Guidance and methods of testing have been given by the IUMS-ICFMH Working Party on Culture Media (Corry et al., 2003).

ALOA™ is now recommended as the first medium in the new ISO Standards which will be published in 2004. However, the ISO/TC 34 SC 9 group concluded at their meeting in Dec. 2002: ALOA™ is recommended for both the detection method EN ISO 11290-1 and for the enumeration method EN ISO 11290-2. For the detection method a further medium, e.g. another chromogenic medium, is allowed. For the enumeration method further discussion is necessary to incorporate other chromogenic plating media. Very recently, some further chromogenic *L. monocytogenes* plating media have been launched (LIMONO-Ident-Agar and OCLA). Their application will depend on the food and the processing conditions to be tested.

The criteria given below should be the basis for the use of any chromogenic plating medium for *Listeria* spp. At present, these media are suitable for:

- detection of *L. monocytogenes* and *L. ivanovii* after 24 h at 36 ± 1 °C
- enumeration after 40–48 h at 36 ± 1 °C
- detection of pathogenic and non-pathogenic *Listeria* spp.

Media which meet these requirements may be recommended and are accepted for use in standard methods.

Since the chromogenic *L. monocytogenes* plating media discussed detect the pathogenic listeriae (*L. monocytogenes* and *L. ivanovii*) directly their designation could also be changed in favour of a new name or an additional description to express this quality, e.g. ''chromogenic culture medium for detection and enumeration of pathogenic *Listeria* spp.''.

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References

Anonymous, 1995. Milk and milk products. Detection of *Listeria monocytogenes*. FIL-IDF Standard, vol. 143A. International Dairy Federation, Brussels.

Anonymous, 1997. Microbiology of food and animal feeding stuffs-horizontal method for the detection and enumeration of *Listeria monocytogenes*: Part 1. Detection Method. International Standard ISO 11290-1. International Organization for Standardization, Geneva.

Anonymous, 1998. Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*: Part 2. Enumeration. International Standard ISO 11290-2. International Organisation for Standardisation, Geneva.

Anonymous, 2002. Ring-trial of the ISO/TC 34 SC 9 group 'Comparison of chromogenic *Listeria monocytogenes* plating media', finished November 15, 2002.

Anonymous, 2003a. Commission regulation (EC) on microbiological criteria for foodstuffs. Draft 6.11.2003, revision no. 7, November 7, 2003.

Anonymous, 2003b. Bacteriological Analytical Manual Online. Guideline for BAM Users on identification of atypical haemolytic Listeria isolates. U.S. Food and Drug Administration. Hypertext updated 2003 May 27. Anthony.hitchins@cfsan.fda.gov.

Baird-Parker, A.C., 1994. Foods and microbiological risks. Microbiology 140, 687–695.

Becker, H., Märtlbauer, E., 2002. Conventional and commercially-available methods in food microbiology. Biotest Bull. 6, 265–319.

Becker, B., Murphy, J., 2000. Vergleichende Untersuchungen zum schnellen Nachweis von *Listeria monocytogenes* mit einem Gensondentest und chromogenem Medium. Poster on the 41.Tagung des Arbeitsgebietes Lebensmittelhygiene der Deutschen Veterinärmedizinischen Gesellschaft.-Kurzfassungen, Vorträge und Poster. Garmisch-Partenkirchen Germany, September 2000.

Becker, B., Schillinger, U., Murphy, J., Holzapfel, W.H., 2001. Schnelle Identifizierung von *Listeria monocytogenes* mittels PCR und eines chromogenen Mediums. Poster on the Symposium Schnellmethoden und Automatisierung in der Lebensmittelmikrobiologie, Lemgo, Germany, July 2001 (Abstracts).

Beumer, R.R., Curtis, G.D.W., 2003. Culture media and methods for the isolation of *Listeria monocytogenes*. In: Corry, J.E.L., Curtis, G.D.W., Baird, R.M. (Eds.), Handbook of Culture Media for Food Microbiology. Elsevier, Amsterdam, pp. 79–90.

Bhagwhat, A.A., 2003. Simultaneous detection of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* strains by real-time PCR. Int. J. Food Microbiol. 84, 217–224.

Buchanan, L.R., Lindquist, R., 2000. Hazard identification and hazard characterization of *Listeria monocytogenes* in ready-to-eat foods. Food and Agricultural Organization of the United Nations and the World Health Organization.

Camilli, A., Goldfine, H., Portnoy, D.A., 1991. *Listeria monocytogenes* mutants lacking phosphatidylinositol-specific phospholipase C are avirulent. J. Exp. Med. 173, 751–754.

Choi, S.W., Hong, C.-H., 2003. Rapid enumeration of *Listeria monocytogenes* in milk using competitive PCR. Int. J. Food Microbiol. 84, 79–85.

Corry, J.E.L., Curtis, G.D.W., Baird, R.M. (Eds.), 2003. Handbook of Culture Media for Food Microbiology. Elsevier, Amsterdam.

Curtis, G.D.W, Lee, W.H., 1995. Culture media and methods for the isolation of *Listeria monocytogenes*. Int. J. Food Microbiol 26, 1–13.

DGHM, 2003. Veröffentlichte mikrobiologische Richt-und Warnwerte zur Beurteilung von Lebensmitteln. Eine Empfehlung der Fachgruppe Lebensmittel-Mikrobiologie und Lebensmittel-Hygiene der Deutschen Gesellschaft für Hygiene und Mikrobiologie, Stand März 2003.

Ermolaeva, S., Karpova, T., Novella, S., Wagner, M., Scortti, M., Tartakovski, I., Vàzquez-Boland, J.A., 2003. A simple method for the differentiation of *Listeria monocytogenes* based on induction of lecithinase activity by charcoal. Int. J. Food Microbiol. 82, 87–94.

European Commision, 1999. Opinion of the scientific committee on veterinary measures relating to public health on *Listeria monocytogenes*. Health and Consumers Protection Directorate-General, Directorate B-scientific health opinions. Unit B3-Management of scientific committees II, September 23, 1999.

Facon, J.-P., Simon, F., 1998. Culture medium for detecting pathogenic bacteria of the genus Listeria and method for identifying

said bacteria. United States Patent US 6,228,606 B1. PCT filed Jul. 10,1998; date of patent May 8, 2001.

Faleiro, M.L., Andrew, P.W., Power, D., 2003. Stress response of *Listeria monocytogenes* isolated from cheese and other foods. Int. J. Food Microbiol. 84, 207–216.

Fitter, S., Heuzenroeder, M., Thomas, C.J., 1992. A combined PCR and selective enrichment method for rapid detection of *Listeria monocytogenes*. J. Appl. Bacteriol. 73, 53–59.

Gouin, E., Mengaud, J., Cossart, P., 1994. The virulence gene cluster of *Listeria monocytogenes* is also present in *Listeria ivanovii,* an animal pathogen, and *Listeria seeligeri*, a non-pathogenic species. Inf. Immun. 62, 3550–3553.

Gracieux, P., Roche, S.M., Pardon, P., Velge, P., 2003. Hypovirulent *Listeria monocytogenes* strains are less frequently recovered than virulent strains on PALCAM and Rapid'L.mono media. Int. J. Food Microbiol. 83, 133–145.

Hechelmann, H., Albert, T., Gareis, M., 2001. Vorkommen und quantitativer Nachweis von *Listeria monocytogenes* in streichfähiger Rohwurst und Rohwurstbrät. Mittgsbl. Bundesanstalt für Fleischforschung 152, 101–107.

Hechelmann, H., Albert, T., Reissbrodt, R., 2002. Characterization of new chromogenic plating media for detection and enumeration of *Listeria monocytogenes*. Poster at Food Micro 2002 Lillehammer, Norway, August, 2002.

Hitchins, A.D., 2002. Critical Steps in detection of *L.monocytogenes* using the FDA BAM culture methodology. Lecture on the 5th Annual Food Pathogen Analysis Conference. St. Pete Beach, Florida, 29th July, 2002.

Hitchins, A.D., 2003. Bacteriological Analytical Manual Online. Chapter 10: Detection and enumeration of *Listeria monocytogenes* in foods. Published by the U.S. Food and Drug Administration, January 2003; hypertex updated May 27th, 2003.

Hoffman, A.D., Wiedmann, M., 2001. Comparative evaluation of culture-and BAX polymerase chain reaction-based detection methods for Listeria spp. and *Listeria monocytogenes* in environmental and raw fish samples. J. Food Prot. 4, 1521–1526.

ISO/TC 34 /SC 9,2002. Report of the meeting of ISO/TC 34/SC 9 "Agricultural and food products—Microbiology". Bangkok, Resolution 192, 2nd to 4th December 2002.

Istafanos, P., James, L., Hunt, J., 2002. Comparison of visual immunoassay and chromogenic culture medium for the presence of *Listeria* spp. in foods. J. AOAC Int. 85, 1201–1203.

Jinneman, K.C., Hunt, J.M., Eklund, C.A., Wernberg, J.S., Sado, P.N., Johnson, J.M., Richter, R.S., Torres, S.T., Ayotte, E., Eliasberg, S.J., Istafanos, P., Bass, D., Kexel-Calabresa, N., Lin, W., Barton, C.N., 2003. Evaluation and interlaboratory validation of a selective agar for using a chromogenic substrate to detect *Listeria monocytogenes* from foods. J. Food Prot. 66, 441–445.

Johansson, T., 1998. Enhanced detection and enumeration of *Listeria monocytogenes* from foodstuffs and food-processing environments. Int. J. Food Microbiol. 40, 77–85.

Johansson, T., Ahola-Luttila, H., Pirhonen, T., Taimisto, A.-M., Haario, H., Laine, M., Salkinoja-Salonen, M., 2000. Improved detection of Listeria monocytogenes in soft mould-ripened cheese. J. Appl. Microbiol. 88, 870–876.

Karpiskova, R., Pejchalova, M., Mokrosova, J., Vytrasova, J., Smuharova, P., Ruprich, J., 2000. Application of a chromogenic medium and the PCR method for the rapid confirmation of *Listeria monocytogenes* in foodstuff. J. Microbiol. Methods 41, 267–271.

King, T., Ferenci, T., Szabo, E.A., 2003. The effect of growth atmosphere on the ability of *Listeria monocytogenes* to survive exposure to acid, proteolytic enzymes and bile salts. Int. J. Food Microbiol. 84, 133–143.

Loessner, M.J., Bell, R.H., Jay, J.M., Shelef, L.A., 1988. Comparison of seven plating media for enumeration of *Listeria* spp. Appl. Environ. Microbiol. 54, 3003–3007.

Notermans, S.H., Dufrenne, J., Leimeister-Wächter, M., Domann, E., Chakraborty, T., 1991. Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic Listeria species. Appl. Environ. Microbiol. 57, 2666–2670.

Ottaviani, F., Ottaviani, M., Agosti, M., 1997a. Differential Agar medium for *Listeria monocytogenes*. "Quimper Froid. Symposium proceedings" P6 A.D.R.I.A. Quimper (F), 16–18.

Ottaviani, F., Ottaviani, M., Agosti, M., 1997b. Esperienza su un agar selettivo differenziale per *Listeria monocytogenes*. Ind. Aliment. 36, 1–3.

Restaino, L., Frampton, E.W., Irbe, R.M., Schabert, G., Spitz, H., H., 1999. Isolation and detection of *Listeria monocytogenes* using fluorogenic and chromogenic substrates for phosphatidylinositol-specific phospholipase C. J. Food Prot. 62, 244–251.

Roczen, D., Knödlseder, M., Friedrich, K., Schabert, G., Spitz, H., Müller, R., Reissbrodt, R., 2003. Development of a new chromogenic *Listeria monocytogenes* plating medium and comparison with three other chromogenic plating media. Poster 048 on the 103. ASM-Meeting in Washington, DC, USA, May 2003.

Schabert, G., Restaino, L., 1998. Novel potentially fluorogenic compounds. European Patent Application EP 0949 266 A1, Date of filing 23.03.1998; date of publication 13.10.1999.

Schuler, S., Becker, B., Murphy, J., Holzapfel, W.H., 2003. Selektiver Schnellnachweis von *Listeria monocytogenes* in geräuchertem Lachs auf zwei chromogenen Medien. Poster on the 5. Fachsymposium Lebensmittelmikrobiologie of the VAAM, 5–8. May 2003, Kloster Seeon, Germany.

Smith, P., Mellors, D., Holroyd, A., Gray, C., 1999. Improved isolation of *Listeria* spp. using a novel specific substrate medium. Poster presentation, 9th Meeting of the Microbiological Methods Innovations Forum. Campden & Chorleywood Food Research Association, Chipping Camden, Gloucestershire, GL55 6LD.

Smith, P., Mellors, D., Holroyd, A., Gray, C., 2001. New chromogenic medium for the isolation of *Listeria* spp. Lett. Appl. Microbiol. 32, 78–82.

Vàzquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., Wehland, J., Kreft, J., 2001. Listeria pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14, 584–640.

Vlaemynck, G., Lafarge, V., Scotter, S., 2000. Improvement of the detection of *Listeria monocytogenes* by application of ALOA, a diagnostic, chromogenic isolation medium. J. Appl. Microbiol. 88, 430–441.