

## The Benefits of ALOA Chromogenic Agar for the Isolation and Identification of *Listeria monocytogenes*

Available exclusively in the UK from Microgen

The integration of differential capabilities into microbiological culture media is a well accepted concept in bacteriology e.g. Lactose in MacConkey Agar and H<sub>2</sub>S in DCA and XLD Agar. Over time, these differential capabilities have often formed the basis of what have become well established identification schemes. However, most of these differential tests are relatively non specific and prone to false positive or negative results.

More recently, chromogenic substrates have proven to be powerful differential agents for the differentiation of specific, closely related organisms in mixed populations e.g. *E. coli* Coliforms (1), *Listeria spp.* and *Salmonella spp.* (2). These substrates are however expensive resulting in the development of culture media which are significantly more costly than conventional media. This higher cost raises the question as to whether this additional expense can be justified.

**Table 1. Comparison of ISO and ALOA (AFNOR Validated Method) for the Isolation and Identification of *L. monocytogenes* from Food Samples.**

ISO Method	ALOA Method
<b>DAY 0</b> 25gm Sample + 225ml Fraser Broth ↓ 24hr at 30°C	<b>DAY 0</b> 25gm Sample + 225ml ½ Fraser Broth ↓ 24hr at 30°C
<b>DAY 1</b> Transfer sample to 224ml Fraser Broth ↓ 24hr at 37°C	<b>DAY 1</b> Subculture 0.1ml to ALOA Agar Plates ↓ 24 hr at 37°C
<b>DAY 2</b> Subculture 0.1ml to Oxford Agar Plates ↓ 24/48 hr at 37°C	<b>DAY 2</b> Examine for colonies resembling <i>Listeria spp.</i> <b>REPORT NEGATIVE RESULTS</b> or confirm genus Perform full biochemical identification Microgen™ <i>Listeria</i> ID ↓ 24hr at 37°C
<b>DAY 3/4</b> Examine for colonies resembling <i>Listeria spp.</i> <b>REPORT NEGATIVE RESULTS</b> or confirm genus Perform full biochemical identification ↓ 24hr at 37°C	<b>DAY 3</b> Read biochemical identification <b>FINAL REPORT</b>
<b>DAY 5/6</b> Read biochemical identification <b>FINAL REPORT</b>	

Recently, ALOA Medium has been introduced for the isolation and differentiation of *Listeria spp.* and *L. monocytogenes*.

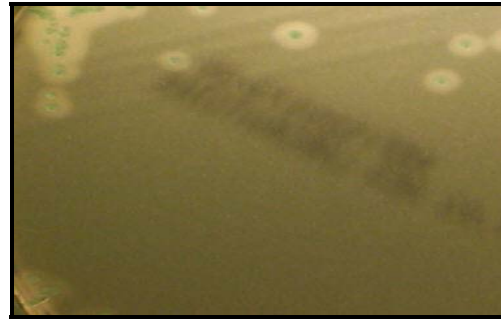
Conventional methods for the isolation and identification of *L. monocytogenes* (3) are expensive and time consuming. ALOA agar has been found to provide a number of functional (sensitivity and specificity), service (sample turn around time) and cost benefits.

## Product Description

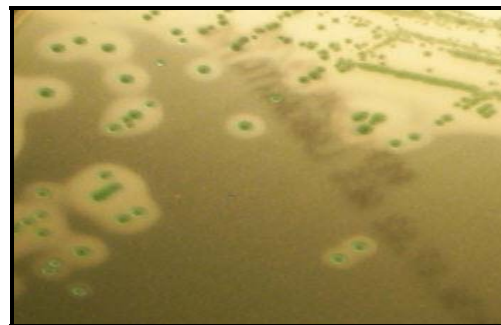
ALOA agar is a pre prepared, selective and differential medium for the isolation of *Listeria spp.* from food samples and for the presumptive identification of *L. monocytogenes*. To minimise the growth of contaminating organisms, lithium chloride and a balanced antimicrobial and antifungal mixture is employed. The incorporation of the chromogenic substrate X-glucoside for the detection of beta-glucosidase demonstrates the presence of *Listeria spp.*, whilst the detection of a specific phospholipase C enzyme produced by pathogenic *Listeria spp.* including *L. monocytogenes* is also achieved. *Listeria spp.* grow on this medium producing blue - green colonies, with pathogenic species (*L. monocytogenes* and *L. ivanovii*) producing similar coloured colonies surrounded by a characteristic opaque halo after 24 hours incubation at 37°C. Non *Listeria spp.* produce white colonies.

The recommended procedure for the use of ALOA Agar, is

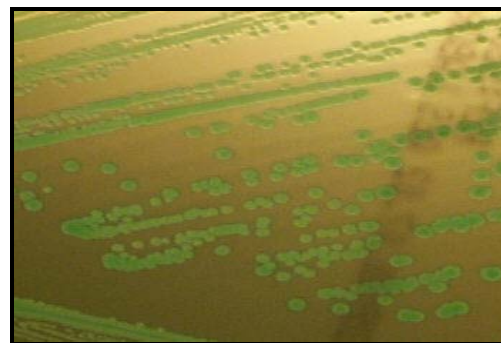
**Figure 1. Typical colonial morphology of selected *Listeria spp.* on ALOA agar plates**



**Typical colonies of *L. monocytogenes***



**Typical colonies of *L. ivanovii***



**Typical colonies of *L. innocua***

shown in Table 1. This procedure is an AFNOR Validated Method (AES 10/3 – 09/00).

## Functional Benefits

A comprehensive investigation into the performance of ALOA was

performed by Vlaemynck (4).

In the first part of this study, pure cultures of 12 different serovars of *L. monocytogenes* were stressed and quantitative counts performed on TSA, ALOA, Oxford and PALCAM Agar Plates. In addition, 50 isolates of *L. monocytogenes*, 50 isolates of

*Listeria spp.*, other than *L. monocytogenes* and 44 strains of potential non *Listeria* competitors belonging to different species were inoculated onto each medium and examined qualitatively.

In the second phase of this study, a total of 208 food samples (dairy and poultry samples) expected to be naturally contaminated with *Listeria spp.* were analysed in accordance with EN ISO 11290-1:1997 with the final enrichment broth being subcultured onto ALOA, Oxford and PALCAM Agar Plates.

In the first study, no differences could be demonstrated in the recovery of *L. monocytogenes* with these 3 media. In addition, no significant reduction in the levels of recovery were noted with any of these selective media when compared to the non selective TSA.

The recovery of non *L. monocytogenes* strains of *Listeria spp.* on ALOA agar was equivalent to Oxford and PALCAM for *L. innocua* and *L. welshimeri*. The recovery of *L. ivanovii*, *L. seeligeri* and *L. grayi* on ALOA was reduced when compared to the less selective Oxford Agar. PALCAM Agar was found to be the most inhibitory towards these 3 species.

All *Listeria spp.* examined produced typical green – blue coloured colonies 1.0 – 2.0mm in diameter after 24 hours incubation, with all serovars of *L. monocytogenes* producing a distinctive opaque halo. Strains of *L.*

*ivanovii* also showed a halo which intensified with further incubation.

Of the non *Listeria* strains examined, only some strains of *Bacillus cereus*, and *Enterococcus faecium* grew on ALOA.

Of the 208 samples examined 36 samples were positive for *L. monocytogenes*. Of these, 31 (86.1%) samples were positive using ALOA, whilst 22 (61.1%) samples were positive using Oxford and PALCAM Agars. In addition, 39 samples were positive on ALOA, Oxford and PALCAM for *Listeria spp.* other than *L. monocytogenes*.

**The authors concluded that one of the major advantages of introducing a medium such as ALOA into *Listeria* isolation protocols was that it improved the detection of colonies highly suggestive of being *L. monocytogenes* in the presence of high numbers of non-pathogenic *Listeria spp.* and other competing organisms.**

### Service Benefits

The service benefits of ALOA medium can be divided into 2 basic categories.

- Test cost, and
- Test turn around time

When *Listeria spp.* are isolated on conventional *Listeria* isolation media, multiple colonies (generally 5) must be selected for identification in an attempt to confirm the presence of *L. monocytogenes*. The enhanced differential capabilities of ALOA

medium allows the more specific targeting of colonies suspected of being *L. monocytogenes*. This ability to specifically target suspect colonies eliminates the need for the performance of the basic screening tests such as catalase, oxidase nitrate and motility as recommended in standard methods (3). Where specific identification of isolates is required, it is recommended that the Microgen™ *Listeria*-ID (MID-67) be employed.

Based on customer surveys performed by Microgen Bioproducts, this ability to specifically target *L. monocytogenes* may decrease the cost of identifications by as much as 60%.

### News Flash

**ISO/TC-34-SC9-DOC-N-599, Report of the Bangkok Meeting, December, 2002.**

**ALOA is now recommended in the DRAFT ISO Standard 11290(May 2002) Microbiology of foods and animal feeding stuffs - horizontal method for detection and enumeration of *L. monocytogenes*.**

The Microgen™ *Listeria*-ID contains all of the substrates recommended in ISO 11290-1 and similar standards including an innovative micro well haemolysin test which provides unequivocal confirmation of *L.*

*monocytogenes* and *L. ivanovii*.

The performance of Microgen™ Listeria-ID has been validated from colonies isolated on ALOA agar.

The ability of ALOA medium to differentiate *L. monocytogenes* from other *Listeria spp.* and the superior differentiation of *Listeria spp.* from non *Listeria spp.* enables the reporting of test results negative for *L. monocytogenes*, 24 – 48 hours earlier than with Oxford or PALCAM agars.

This ability to report results earlier offers many advantages to both commercial testing laboratories competing for business and also to manufacturer operated testing laboratories. The ability to clear products for release earlier, particularly in the often short shelf life products where *Listeria* may be found, offers major advantages to manufacturers.

## Do I Need to Identify *L. monocytogenes*?

**YES.** Although the development of the opaque halo around colonies of *L. monocytogenes* is highly characteristic, *L. ivanovii* also produce the specific phospholipase C responsible for this halo. Therefore, in order to accurately report the presence of *L. monocytogenes*, specific identification must be performed using Microgen™ Listeria-ID (MID-67) which is currently undergoing AFNOR validation.

## References

1. Geissler K, Manafi M, Amoros I, Alonso JL . Quantitative determination of total coliforms and *Escherichia coli* in marine waters with chromogenic and fluorogenic media. J Appl Microbiol 2000; **88**(2):280-5

2. Perry JD, Ford M, Taylor J, Jones AL, Freeman R, Gould FK. ABC medium, a new chromogenic agar for selective isolation of *Salmonella spp.* J Clin Microbiol 1999 Mar; **37**(3):766-8
3. ISO Standard 11290-1 (Part 1 + 2) Microbiology of foods and animal feeding stuffs - horizontal method for detection and enumeration of *L. monocytogenes*
4. G.Vlaemynck G, Lafrage V. and S.Scotter. Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. J. App. Microbiol 2000; **88**: 430 – 441.

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