Comparison of BBL™ CHROMagar™ Listeria to Currently Recommended Media for the Isolation of Listeria monocytogenes from Food Sources

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INTRODUCTION

Listeria monocytogenes is the causative agent of a serious foodborne illness. About 2,500 cases of listeriosis are reported yearly in the United States with a mortality rate of about 20%. Foodborne listeriosis is of particular concern for immunocompromised patients, pregnant women, neonates and the elderly.

The isolation and identification of Listeria monocytogenes is of importance, particularly in food microbiology for some ready-to-eat foods, dairy, and poultry products. FDA and USDA/FSIS recommends culturing samples in an enrichment broth followed by subculture to two different differential, selective agars. Suspected isolates are subcultured on non-selective agar and identified using biochemical or kit tests or using genus or species specific ELISA or DNA probes. Six species of Listeria need to be differentiated with complete characterization using at least five colonies from each media type. L. monocytogenes is the only species consistently associated with human illness.

BBL™ CHROMagar™ Listeria (CL) is a selective and differential medium for the isolation and presumptive identification of L. monocytogenes in food. CL is composed of a nutritive base and selective supplements. A proprietary blend of chromogen and phospholipid offers differentiation of L. monocytogenes from other organisms.

This study compares the sensitivity and specificity of each media at 24 and 48 hours of incubation. CL was evaluated against the currently recommended FDA and USDA media PALCAM Medium (PAL), Oxford Agar (OX), Modified Oxford Agar (MOX), and LPM Agar (LPM) for the isolation and presumptive identification of L. monocytogenes in food. This study compares the sensitivity and specificity of each media at 24 and 48 hours of incubation.

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MATERIALS AND METHODS

MEDIA
- BBL™ CHROMagar™ Listeria (BBL)
- PALCAM Medium (Difco, Cat. No. 263620 and 263710)
- Oxford Agar (Difco, Cat. Nos. 222530 and 211755)
- Modified Oxford Agar (Remel, ref 01613)
- LPM Agar (Difco, Cat. No. 222120)
- Trypticase™ Soy Agar with 5% Sheep Blood, TSA II™, (BBL™ Cat. No. 221261)

TEST STRAINS (Table A)
- Listeria monocytogenes: 6 ATCC™, 42 reference/clinical/food
- Listeria species, not L. monocytogenes, 5 ATCC, 21 clinical/food
- Other (Bacillus sp, enterococci, gram negative bacilli, staphylococci, yeast and fungi): 25 ATCC

METHOD
Each test strain was inoculated on the five media listed. Inocula were prepared in sterile deionized water from fresh overnight cultures and streaked in four quadrants for isolation.

L. monocytogenes and Listeria species were inoculated at a concentration of 10^3 CFU per plate: other organisms were inoculated at 10^5 CFU per plate. All media were incubated aerobically at 35°C, examined at 24 and 48 hours and compared to TSA II (a non-selective control) for recovery of L. monocytogenes and ability to differentiate L. monocytogenes from other organisms (by colony coloration). Colonies of L. monocytogenes are blue with a white halo on CL, gray-green with black precipitate on PAL, black with black precipitate on OX and MOX and light blue or white on LPM. Colonies of Listeria species, not L. monocytogenes or L. ivanovii, are blue without a halo on CL, gray-green with black precipitate on PAL, black with black precipitate on OX and MOX and light blue to white on LPM. Most other organisms exhibit complete to partial inhibition on all media. Recommended incubation temperature, time and expected results are summarized in the following chart:

<table>
<thead>
<tr>
<th>MEDIA</th>
<th>CATALOG NUMBER</th>
<th>INC. TEMP</th>
<th>INC. TIME</th>
<th>EXPECTED RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPM Agar Base</td>
<td>Difco: 222120</td>
<td>35 +/- 2</td>
<td>18–48 hours</td>
<td>Listeria are a white, gray to blue color with a ground glass appearance.</td>
</tr>
<tr>
<td>LPM Supplement</td>
<td>Difco: 201556</td>
<td>35 +/- 2</td>
<td>18–48 hours</td>
<td></td>
</tr>
<tr>
<td>PALCAM</td>
<td>Difco: 263620</td>
<td>35 +/- 2</td>
<td>24–48 hours</td>
<td>Listeria are a gray-green with a black precipitate.</td>
</tr>
<tr>
<td>Palcam Antimicrobial Supp.</td>
<td>Difco: 263620</td>
<td>35 +/- 2</td>
<td>24–48 hours</td>
<td></td>
</tr>
<tr>
<td>Oxford Medium Base</td>
<td>Difco: 222530</td>
<td>35 +/- 2</td>
<td>18–48 hours</td>
<td>Listeria are black with a black precipitate.</td>
</tr>
<tr>
<td>Oxford Antimicrobial Supp.</td>
<td>Difco: 263710</td>
<td>35 +/- 2</td>
<td>18–48 hours</td>
<td></td>
</tr>
<tr>
<td>Remel MOX</td>
<td>Remel, Ref 01613</td>
<td>35 +/- 2</td>
<td>18–48 hours</td>
<td>Listeria are black with a black precipitate.</td>
</tr>
<tr>
<td>CHROMagar™ Listeria</td>
<td>BBL</td>
<td>35 +/- 2</td>
<td>24–48 hours</td>
<td>Listeria monocytogenes are medium to large, blue with white halo. L. ivanovii are small, blue with white halo. Other Listeria species, if growth occurs, are blue without halo.</td>
</tr>
</tbody>
</table>
**RESULTS AND DISCUSSION**

**Listeria monocytogenes:**

Detection of Listeria monocytogenes at 24 hours was best on CL and MOX. Detection improved at 48 hours on all media with CL and LPM detecting 100% of the isolates. CHROMagar Listeria is the only media evaluated which provides presumptive identification of L. monocytogenes. Medium to large blue colonies with white halos on CHROMagar Listeria can be presumptively identified as L. monocytogenes. L. ivanovii also produces blue colonies with a white halo on CL, but produces a small to pinpoint colony.

**Listeria species not monocytogenes:**

Each media was evaluated for ability to differentiate L. monocytogenes from Listeria non-monocytogenes species. The Listeria species which resembled L. monocytogenes on PAL, OX, MOX, LPM and CL would require biochemical testing to differentiate or rule out L. monocytogenes. Only L. ivanovii produced a blue colony with white halo on CL; however, the colony size of L. ivanovii is smaller permitting differentiation from L. monocytogenes. No other Listeria species produce blue colonies with white halos on CL.

**Other organisms:**

Many of the organisms tested in the “non-Listeria group” were inhibited. Those that did grow did not produce colony growth that would have been considered suspicious or presumptive for L. monocytogenes on any media, with the exception of LPM. At 48 hours, 4 organisms (3 Bacillus species and 1 S. aureus) were recovered on LPM with the expected colony morphology of L. monocytogenes. The overall specificity at 48 hours was 57% for PAL, 57% for OX, 49% for MOX, 49% for LPM, and 90% for CL.

**Discussion:**

Procedures for Listeria analysis of food products recommend taking at least five typical colonies from each media for identification. Five colonies are necessary since more than one Listeria species may be present in the same sample.

The improved specificity of CL over the other media tested in this study, would result in fewer colonies resembling the appearance of L. monocytogenes and need for subsequent identifications. Listeria species, non-monocytogenes produced colonies typical of L. monocytogenes on PAL, OX, MOX, and LPM and would require additional tests to rule out the presence of L. monocytogenes.
CONCLUSIONS

- All the media tested in this study had good sensitivity and were able to detect *L. monocytogenes* within 48 hours.
- BBL CHROMagar Listeria offers an advantage over PALCAM, Oxford and LPM by providing a reliable, presumptive identification of *L. monocytogenes* at 24 hours.
- BBL CHROMagar Listeria and LPM were the only media which detected 100% of the *L. monocytogenes* at 48 hours.
- BBL CHROMagar Listeria demonstrated superior specificity (92% at 24 and 90% at 48 hours).
- BBL CHROMagar Listeria is sensitive and offers increased specificity, providing timely, accurate results and eliminating the need for most supplemental testing for presumptive identification of *L. monocytogenes*. 