

# BD BBL™ CHROMagar™ Listeria



\*See footnote below

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## INTENDED USE

**BBL™ CHROMagar™ Listeria** is a selective medium for the isolation, differentiation and identification of *Listeria monocytogenes* and *L. ivanovii* from food and environmental samples.

**BBL CHROMagar Listeria** has been validated by the AOAC™ Research Institute under the Performance Tested Methods<sup>SM</sup> Program for the analysis of raw ground beef, smoked salmon, lettuce and Brie cheese when using FDA BAM, USDA FSIS, AOAC and ISO methods<sup>1-4</sup> with no confirmatory biochemical tests required for the identification of *Listeria monocytogenes* / *L. ivanovii*.

Confirmatory testing of isolates from food matrices other than those that have been validated, and from environmental samples, is recommended.

## SUMMARY AND EXPLANATION

Listeriosis is a foodborne illness caused by *L. monocytogenes*. It is of particular concern for immunocompromised patients: cancer, HIV, pregnant women, neonates and the elderly. Because of the severity of the disease, 20 deaths per 100 cases, listeriosis is a serious public health and agri-food industry concern. Illness caused by *L. monocytogenes* has been associated with deli meats, poultry, soft cheeses, ready-to-eat seafood, smoked fish, hot dogs, salad greens and inadequately or unpasteurized milk.<sup>5,6</sup> *L. ivanovii*, rarely found in foods, is pathogenic to animals and some cases of human listeriosis have been associated with this organism.<sup>7</sup>

**BBL CHROMagar Listeria** is intended for the isolation, differentiation and identification of *L. monocytogenes* and *L. ivanovii* based on the formation of blue-green colonies surrounded by an opaque, white halo. The addition of a chromogenic and a phospholipid substrate in the medium facilitates the detection and differentiation of *L. monocytogenes* and *L. ivanovii* from other *Listeria* species and organisms.

An advantage **BBL CHROMagar Listeria** has over recommended traditional media, such as Modified Oxford and Oxford, is the ability to distinguish *L. monocytogenes* and *L. ivanovii* from other *Listeria* species. This facilitates the detection of *L. monocytogenes* / *L. ivanovii* in the presence of other *Listeria* species and other bacterial flora that may be present in a sample, thereby minimizing the risk of not detecting *L. monocytogenes* or *L. ivanovii*.

Studies using **BBL CHROMagar Listeria** for testing a variety of food and environmental samples demonstrated 99-100% sensitivity and 100% specificity with a detection level of 1-18 CFU/25 g. See Performance Characteristics.

## PRINCIPLES OF THE PROCEDURE

**CHROMagar Listeria** was originally developed by A. Rambach, CHROMagar, Paris, France. **BD**, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar Listeria** prepared plated medium. Specially selected **Difco™** peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and fungi. The chromogen is a chromogenic substrate that produces a blue-green colored compound when hydrolyzed by an enzyme specific to *Listeria* species. A specific enzyme found in *L. monocytogenes* and *L. ivanovii* acts upon the phospholipid substrate in **BBL CHROMagar Listeria** producing an opaque, white halo around the blue-green colonies. The growth of a blue-green colony with well-defined edges surrounded by an opaque, white halo is presumptive for *L. monocytogenes* or *L. ivanovii* on **BBL CHROMagar Listeria**.

## REAGENTS

### BBL CHROMagar Listeria

Approximate Formula\* Per Liter of Purified Water

Peptones and Meat Extracts .....	23.0 g
Chromogen Mix .....	17.5 g
Agar .....	15.0 g
Salt .....	5.0 g
Inhibitory Agents .....	0.04 g

\*Adjusted and/or supplemented as required to meet performance criteria.

### Warnings and Precautions:

For Laboratory Use.

If excessive moisture is observed, invert the bottom over the off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Protect from light during drying. See Storage Instructions.

Pathogenic microorganisms, including *Listeria monocytogenes*, may be present in food samples. Observe aseptic techniques and established precautions<sup>8,9</sup> against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in the dark at 2 to 8°C in original sleeve wrapping and original cardboard box until time of inoculation. Plates may be used until the expiration date. Minimize the exposure of **BBL CHROMagar Listeria** to light both before and during incubation, as light may destroy the chromogens.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

## PROCEDURE

**Materials Provided:** **BBL CHROMagar Listeria**

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

### Sample Collection & Preparation

Follow appropriate U.S. Food and Drug Administration (FDA), U.S. Department of Agriculture (USDA), Health Canada or ISO Standards for detection and enumeration methods as required for details of sample preparation, cultivation in enrichment broth, and handling procedure guidelines appropriate to sample type and geographic location.

### Test Procedure:

Allow medium to warm to room temperature before inoculation. Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the incubated enrichment broth sample onto a **BBL CHROMagar Listeria** plate and streak for isolation. Incubate plates aerobically at 35 ± 2°C in an inverted position (agar-side up) for 24 h. Do not incubate in CO<sub>2</sub>. If negative, reincubate for an additional 24 h to report final results.

### User Quality Control

Examine plates for signs of deterioration as described under "Product Deterioration." Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

Test Strain	Expected Results
<i>Listeria monocytogenes</i> ATCC™ 19114	Growth: blue-green colony with an opaque, white halo
<i>Listeria innocua</i> ATCC 33090	Growth: blue-green colony without a halo
<i>Proteus mirabilis</i> ATCC 12453	No growth

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's Quality Control procedures.

## RESULTS

After proper incubation, read plates against a white background with good lighting. A blue-green colony surrounded by an opaque, white halo on **BBL CHROMagar Listeria** is indicative of *L. monocytogenes* / *L. ivanovii*. Other *Listeria* species will produce blue-green colonies without a halo. Gram-negative organisms are inhibited. Gram-positive organisms, other than *Listeria* species, will either be inhibited or produce white colonies.

## LIMITATIONS OF THE PROCEDURE

**CHROMagar Listeria** cannot differentiate *L. monocytogenes* from *L. ivanovii* based on colony color or halo formation. Supplemental tests, such as hemolysis, xylose, rhamnose and CAMP or commercially available AOAC-RI approved *Listeria* identification biochemical test kits are necessary for differentiation of *L. monocytogenes* and *L. ivanovii*.

Incubation in CO<sub>2</sub> may adversely affect the recovery of *Listeria* species.

## PERFORMANCE CHARACTERISTICS

**BBL CHROMagar Listeria (CL)** was validated by the AOAC Research Institute under the Performance Tested Methods Program.<sup>10</sup> It was evaluated for the detection of *Listeria monocytogenes* in raw ground beef, smoked salmon, lettuce and Brie cheese. The recovery of *L. monocytogenes* on CL was compared to the FDA BAM, USDA FSIS, AOAC and ISO reference plated media using the recommended pre-enrichments and selective enrichments. Of the 265 food samples tested, 140 were tested using BAM, USDA, or AOAC methods and 125 were tested using ISO methods. **BBL CHROMagar Listeria** produced a sensitivity of 99.3% and a specificity of 100% as compared to the reference methods for all food matrices. No false negatives were found in testing the food matrices. No statistical difference was found in recovery using the **BBL CHROMagar Listeria** method compared to the reference plated media based on Chi square analysis. Known isolates were evaluated and CL had a sensitivity and specificity of 100%. The results of these studies demonstrate **BBL CHROMagar Listeria** is an effective medium for the recovery and detection of *Listeria monocytogenes* in raw ground beef, smoked salmon, lettuce and Brie cheese using FDA BAM, USDA FSIS, AOAC and ISO methods. See Table 1 for a summary of validation method comparison study results.

\*PRODUCER-SUPPLIED SAMPLES OF THIS TEST KIT MODEL WERE INDEPENDENTLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AND WERE FOUND TO PERFORM TO THE PRODUCER'S SPECIFICATIONS AS STATED IN THE TEST KIT'S DESCRIPTIVE INSERT. THE PRODUCER CERTIFIES THIS KIT CONFORMS IN ALL RESPECTS TO THE SPECIFICATIONS ORIGINALLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AS DETAILED IN *Performance Tested Methods*<sup>SM</sup> CERTIFICATE NUMBER 060501.

Table 1. Summary of Method Comparison Testing of BBL CHROMagar Listeria Compared to Reference Media

Food Matrices	Method	Level	MPN/g	Total Samples	Total Positive	Reference Positive	CL Positive	P value	Method Agreement*
Raw Ground Beef	USDA	Natural contamination	0.094-0.43	40	18	15	18	Not Significant	92.5%**
Raw Ground Beef	ISO	Low	0.061	20	11	11	10	Not Significant	96.0%
		Control	<0.003	5	0	0	0		
Smoked Salmon	FDA	Low	0.023-0.15	40	37	37	37	Not Significant	100%
		Control	<0.003	10	0	0	0		
Smoked Salmon	ISO	Low	0.240-0.75	40	30	30	30	Not Significant	100%
		Control	<0.003	10	0	0	0		
Lettuce	FDA	Low	0.093	20	17	17	17	Not Significant	100%
		Control	<0.003	5	0	0	0		
Lettuce	ISO	Low	0.093	20	9	9	9	Not Significant	100%
		Control	<0.003	5	0	0	0		
Brie Cheese	AOAC	Low	0.0036	20	9	9	9	Not Significant	100%
		Control	<0.003	5	0	0	0		
Brie Cheese	ISO	Low	0.0043	20	19	19	19	Not Significant	100%
		Control	<0.003	5	0	0	0		

\* Represents confirmed identifications that were equivalent between CL and confirmed positive and negative cultures.

\*\* Three additional positive samples were detected on the test (CL) method for raw ground beef using USDA method.

In the testing of raw ground beef and smoked salmon using ISO method 11290, time to recovery differences were observed for ALOA<sup>11</sup> and BBL CHROMagar Listeria. Twenty-seven positive samples were recovered on BBL CHROMagar Listeria after 24 h of incubation from the primary (Half Fraser) and secondary (Fraser) broths. Three additional positive samples were detected following 48 h of incubation. In contrast, three positive samples were detected on ALOA from the primary and secondary broths after 24 h, with an additional 23 detected following 48 h of incubation. This difference is not reflected in the Chi-square analysis, since the study compared both reference media, Oxford and ALOA, to that of BBL CHROMagar Listeria using the reference procedure for examining each medium at 24 and 48 h. These data are summarized in Table 2.

Table 2. Time to Recovery for BBL CHROMagar Listeria and ALOA

Media (Enrichment/Plate)	Raw Ground Beef n=20		Smoked Salmon n=20	
	Positive at 24 h	Additional Positives at 48 h	Positive at 24 h	Additional Positives at 48 h
Half Fraser/ALOA	1	2	0	1
Half Fraser/ BBL CHROMagar Listeria	3	0	2	3
Fraser/ALOA	1	8	1	12
Fraser/ BBL CHROMagar Listeria	9	0	13	0

In a university study, 200 food samples of 50 types were processed for the isolation and identification of *L. monocytogenes* using 50 natural and 150 spiked samples (100 spiked with *L. monocytogenes* and 50 with other *Listeria* species). Vegetables, milk and milk products, meat, seafood, poultry, ready-to-eat meats and mushrooms were tested. Testing utilized *Listeria* Enrichment and Fraser broths using AOAC and Health Canada reference methods.<sup>3,12</sup> All positive Fraser broths were plated to BBL CHROMagar Listeria, Oxford, Modified Oxford and PALCAM media and a nonselective sheep blood agar plate for comparison. Confirmatory testing was performed on suspect colonies. Overall, BBL CHROMagar Listeria had 100% (2/2) sensitivity and 100% (48/48) specificity when testing natural samples. In samples spiked with *L. monocytogenes*, BBL CHROMagar Listeria detected (99/100) for a sensitivity of 99%. BBL CHROMagar Listeria was able to differentiate four of five *Listeria* species in the spiked samples. Only *L. ivanovii* produced results similar to *L. monocytogenes*.

In a second university laboratory, 63 poultry and environmental samples were processed using the USDA/FSIS *Listeria* isolation method. UVM and Fraser enrichment broths were used. Samples were plated and compared on Modified Oxford and BBL CHROMagar Listeria media. A total of 11 samples were positive for *L. monocytogenes*, with one sample positive for a 4b serotype. All 11 samples exhibited colonies positive for *L. monocytogenes* on BBL CHROMagar Listeria. These colonies were confirmed by PCR testing. BBL CHROMagar Listeria was 100% (11/11) sensitive and 100% (52/52) specific in the detection and differentiation of *L. monocytogenes*. Modified Oxford medium was 100% (11/11) sensitive in the recovery of *L. monocytogenes*, but lacked the ability to differentiate *L. monocytogenes* from other *Listeria* species. Eighteen of the 63 samples exhibited presumptive positive colonies on Modified Oxford medium that required confirmatory testing.

In an internal study, spiked hot dog samples were tested according to the FDA/BAM method. A low inoculum level of 1-5 CFU/25 g and a high inoculum level of 10-50 CFU/25 g were prepared and tested. Sixteen *L. monocytogenes* and 9 *Listeria* species were tested. Recovery on BBL CHROMagar Listeria was compared to Oxford, Modified Oxford and PALCAM media. BBL CHROMagar Listeria recovered 100% (16/16) of *L. monocytogenes* from each inoculum level.<sup>10</sup>

#### AVAILABILITY

Cat. No.	Description
215085	BBL™ CHROMagar™ Listeria, Pkg. of 20 plates
290720	Difco™ Buffered Listeria Enrichment Broth Base, 500 g
222330	Difco™ UVM Modified Listeria Enrichment Broth, 500 g
211767	Difco™ Fraser Broth Base, 500 g
211742	Difco™ Fraser Broth Supplement, 6 x 10 mL
218105	Difco™ Buffered Peptone Water, 500 g

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