

PERFORMANCE TESTED METHODSSM

AOAC RESEARCH INSTITUTE NEWS

BBLTM CHROMagarTM Salmonella and Listeria Granted PTM Status



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Performance-Based
Validation

BD Diagnostics-Diagnostic Systems

BBLTM CHROMagarTM Salmonella

PTM Status: February 22, 2005

Certificate No.: 020502

BBLTM CHROMagarTM Salmonella is a chromogenic selective and differential medium for the presumptive identification of *Salmonella* species (Figure 1). It is available as a ready-to-use prepared plate medium (BBLTM CHROMagarTM Salmonella) or as a dehydrated culture medium (DifcoTM CHROMagarTM Salmonella).

An expert independent laboratory tested BBL CHROMagar Salmonella to evaluate recovery of *Salmonella* compared to the reference U.S. Department of Agriculture-Food Safety Inspection Service (USDA/FSIS), U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (FDA/BAM), and International Organization for Standardization (ISO) media. BBL CHROMagar Salmonella was found to accurately detect the presence of *Salmonella* in a variety of food types, including raw ground beef, raw chicken, raw fish, lettuce, and shell eggs.

Principle of the Test

BBL CHROMagar Salmonella permits the detection and presumptive identification of *Salmonella* species through the incorporation of specific chromogenic substrates and inhibitory agents. *Salmonella* species produce mauve (rose to purple colonies) that are easily differentiated from other bacteria, including coliforms, which may resemble *Salmonella* on other traditional media. Other bacteria are inhibited or produce colorless, tan, white, or blue/green colonies.

Method Validation

An independent laboratory using naturally contaminated and inoculated foods evaluated BBL CHROMagar Salmonella. The prepared plates and plates made from the dehydrated culture medium were compared to Bismuth Sulfitte Agar, Hektoen Enteric Agar, and Xylose Lysine Desoxycholate Agar according to the FDA/BAM method, and compared to Brilliant Green Sulfa Agar and Xylose Lysine TergitolTM 4 Agar according to the USDA/FSIS method. Prepared plates were also evaluated compared to Xylose Lysine Desoxycholate Agar following ISO 6579.

Results of the independent analysis are presented in Tables 1 and 2. BBL CHROMagar Salmonella prepared plates performed as well as the reference media in all the food samples with 100% agreement for each of the three methods. The dehydrated medium also performed as well as the reference media in all the food samples with 100% agreement with the FDA/BAM and

USDA/FSIS reference media.

A total of 127 strains of *Salmonella*, representing 52 different serovars, were inoculated using standard methods onto BBL CHROMagar Salmonella medium (prepared plates and dehydrated medium). Both formats produced identical results. Positive results were obtained for 123 strains. One of the isolates, which failed to grow on BBL CHROMagar Salmonella, was inhibited by the Tetrathionate Broth and was recovered on BBL CHROMagar Salmonella when tested using a nonselective enrichment broth. The overall sensitivity of the media was 96.8%.

To evaluate the ability of BBL CHROMagar Salmonella to differentiate *Salmonella* spp. from non-*Salmonella* strains, a total of 65 isolates, representing 38 different bacteria and yeast, were tested using a nonselective broth. The test battery included 17 *Citrobacter freundii*, two *Proteus mirabilis*, one *P. vulgaris*, four *Shigella* species, and other coliforms since these often produce colonies suggestive of *Salmonella* on traditional media used for recovery of *Salmonella* species. The test strains and results are presented in Table 3. Sixty-one isolates, including all the *C. freundii*, *Proteus*, and *Shigella* isolates, produced negative results. The four strains that produced colonies suggestive of *Salmonella* were *Hafnia alvei* (one of two), *Candida tropicalis* (one of one), and *Pseudomonas aeruginosa* (two of three). The overall specificity of the media was 94% when tested using no selective enrichment steps.

BBL CHROMagar Salmonella was evaluated for consistency of the manu-

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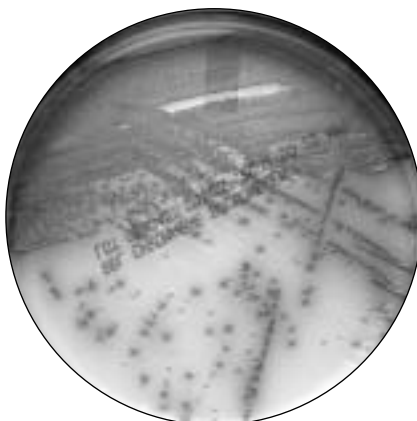


Figure 1. BBL CHROMagar Salmonella with *Salmonella* and *Citrobacter freundii*.

BBL™ CHROMagar™ Salmonella and Listeria Granted PTM Status

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Table 1. BBL CHROMagar Salmonella detection of *Salmonella* species in food matrixes compared to reference media following USDA and FDA methods

Food	Inoculum level	MPN ^a /25 g	No. of samples	BAM or USDA method No. positive	BBL CHROMagar Salmonella (prepared plates) No. positive	BBL CHROMagar Salmonella (dehydrated) No. positive	Chi square value	Method agreement, %
Raw chicken	Naturally contaminated	0.23	20	16	16	16	0.00	100
Raw ground beef	Low	0.043	20	17	17	17	0.00	100
	Control	<0.003	5	0	0	0	0.00	100
Raw fish	Low	0.023	20	18	18	18	0.00	100
	Control	<0.003	5	0	0	0	0.00	100
Lettuce	Low	0.023	20	18	18	18	0.00	100
	Control	<0.003	5	0	0	0	0.00	100
Shell eggs	Low	0.093	20	11	11	11	0.00	100

^a MPN = Most probable number. Calculated by examining triplicate samples of 100, 10, 1, and 0.1 g from each spiked level.

Table 2. BBL CHROMagar Salmonella detection of *Salmonella* species in food matrixes compared to reference media following ISO method

Food	Inoculum level	MPN ^a /25 g	No. of samples	ISO reference method No. positive	BBL CHROMagar Salmonella (prepared plates) No. positive	Chi square value	Method agreement, %
Raw chicken	Naturally contaminated	0.23	20	16	16	0.00	100
Raw ground beef	Low	0.0036	20	17	17	0.00	100
	Control	<0.003	5	0	0	0.00	100
Raw fish	Low	0.0036	20	9	9	0.00	100
	Control	<0.003	5	0	0	0.00	100
Lettuce	Low	0.15	20	19	19	0.00	100
	Control	<0.003	5	0	0	0.00	100
Shell eggs	Low	0.23	20	19	19	0.00	100
	Control	<0.003	5	0	0	0.00	100

^a MPN = Most probable number. Calculated by examining triplicate samples of 100, 10, 1, and 0.1 g from each spiked level.

factured lots, ruggedness, and stability. Three lots each of the prepared plate and plates prepared from the dehydrated culture medium were evaluated for consistency and stability by testing replicates over the shelf life of the products. The reproducibility rate for both formats was 100%. Ruggedness testing demonstrated that both formats could tolerate deviations in incubation temperature over a range of 32–38°C and incubation time of 14–60 hours. When both the incubation time

(14 hours) and temperature (32°C) are at the lower range, positive reactions may be delayed for some strains, unless either the incubation time is extended or the temperature increased. The package insert recommends incubating 24-hour negative cultures for an additional 48 hours prior to reporting as negative.

Conclusions

The BBL CHROMagar Salmonella results (prepared plates and dehydrated

medium) from the AOAC Research Institute (AOACRI) *Performance Tested Method*SM (PTM) Program demonstrated 100% agreement with the reference FDA/BAM and USDA/FSIS plating media. In addition, the BBL CHROMagar Salmonella prepared plates had 100% agreement with ISO plating medium. *Salmonella* species are easily recognized on BBL CHROMagar Salmonella, and potentially false-positive strains such as *C. freundii* are easily differentiated.

The results of this study demonstrate

Table 3. Exclusivity test strains

Total No. of strains tested	Organism	BBL CHROMagar Salmonella result (prepared plates)	BBL CHROMagar Salmonella result (dehydrated)
1	<i>Bacillus cereus</i>	Negative	Negative
1	<i>Candida albicans</i>	Negative	Negative
1	<i>Candida glabrata</i>	Negative	Negative
1	<i>Candida tropicalis</i>	Positive	Positive
17	<i>Citrobacter freundii</i>	Negative	Negative
1	<i>Citrobacter koseri</i>	Negative	Negative
1	<i>Edwardsiella tarda</i>	Negative	Negative
1	<i>Enterobacter aerogenes</i>	Negative	Negative
1	<i>Enterococcus faecalis</i>	Negative	Negative
1	<i>Enterococcus faecium</i>	Negative	Negative
7	<i>Escherichia coli</i>	Negative	Negative
1	<i>Escherichia coli</i> DH5	Negative	Negative
1	<i>Escherichia coli</i> O157	Negative	Negative
1	<i>Escherichia vulneris</i>	Negative	Negative
2	<i>Hafnia alvei</i>	1 Positive and 1 negative	1 Positive and 1 negative
1	<i>Klebsiella oxytoca</i>	Negative	Negative
2	<i>Klebsiella pneumoniae</i>	Negative	Negative
1	<i>Listeria monocytogenes</i>	Negative	Negative
1	<i>Microbacterium lacticum</i>	Negative	Negative
1	<i>Micrococcus luteus</i>	Negative	Negative
1	<i>Morganella morganii</i>	Negative	Negative
2	<i>Proteus mirabilis</i>	Negative	Negative
1	<i>Proteus vulgaris</i>	Negative	Negative
1	<i>Providencia rettgeri</i>	Negative	Negative
1	<i>Providencia stuartii</i>	Negative	Negative
4	<i>Pseudomonas aeruginosa</i>	2 Positive and 2 negative	2 Positive and 2 negative
1	<i>Serratia liquefaciens</i>	Negative	Negative
1	<i>Serratia marcescens</i>	Negative	Negative
1	<i>Shigella boydii</i>	Negative	Negative
1	<i>Shigella dysenteriae</i>	Negative	Negative
1	<i>Shigella flexneri</i>	Negative	Negative
1	<i>Shigella sonnei</i>	Negative	Negative
1	<i>Staphylococcus aureus</i>	Negative	Negative
1	<i>Streptococcus pneumoniae</i>	Negative	Negative
1	<i>Streptococcus pyogenes</i>	Negative	Negative
1	<i>Streptococcus sanguis</i>	Negative	Negative
1	<i>Streptococcus salivarius</i>	Negative	Negative

that BBL CHROMagar Salmonella prepared plates and Difco CHROMagar Salmonella dehydrated culture medium are effective for the isolation and presumptive identification of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce, and shell eggs when compared to any of the three reference methods plated media. This medium represents an approved alternative to

current standard reference plated media for the foods tested. ■

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BBL™ CHROMagar Listeria
PTM Status: June 16, 2005
Certificate No.: 060501

BBL™ CHROMagar™ Listeria is a ready-to-use selective plated medium for the isolation, differentiation, and identification of *Listeria monocytogenes* and *L. ivanovii*

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BBL™ CHROMagar™ Salmonella and Listeria Granted PTM Status

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(Figure 2). BBL CHROMagar Listeria was validated and found to perform equivalent to or better than the reference media for the identification of *L. monocytogenes* in a variety of foods, including raw ground beef, smoked salmon, lettuce, and Brie cheese when FDA/BAM, USDA/FSIS, AOAC, and ISO methods are used.

Principles of the Test

BBL CHROMagar Listeria contains specifically selected peptones to supply nutrients, which facilitate the recovery and isolation of *L. monocytogenes* and *L. ivanovii*. The addition of chromogenic and phospholipid substrates in the medium facilitates the detection and differentiation of *L. monocytogenes* and *L. ivanovii* from other *Listeria* species and organisms. Selective agents inhibit the growth of Gram-negative organisms, yeast, and fungi. An enzyme specific to *Listeria* species hydrolyzes the chromogenic substrate, producing a blue-green colored colony. A specific enzyme found in *L. monocytogenes* and *L. ivanovii* acts upon a phospholipid substrate producing an opaque, white halo around the colony. Confirmatory tests, such as hemolysis, xylose, rhamnose, and CAMP, are necessary to differentiate *L. monocytogenes* and *L. ivanovii*. Growth of a blue-green colony with well-defined edges surrounded by an opaque, white halo is diagnostic for *L. monocytogenes* and *L. ivanovii*.

Method Validation

BBL CHROMagar Listeria was validated by the AOACRI under the PTM Program for testing raw ground beef, smoked salmon, lettuce, and Brie cheese when FDA/BAM, USDA/FSIS, AOAC, and ISO methods were used. Under this program, both internal (BD Diagnostics) and external expert laboratory studies were conducted using naturally contaminated and seeded food samples.

The results from the internal and external method comparison studies using BBL CHROMagar Listeria are presented in Table 4. The reference

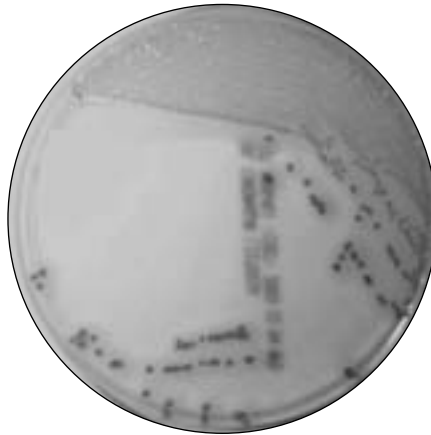


Figure 2. BBL CHROMagar Listeria with *Listeria monocytogenes*.

media detected 147/150 (98.0%) positive samples, and BBL CHROMagar Listeria detected 149/150 (99.3%) positive samples. Based on Chi square analysis, no significant differences ($p < 0.05$) were found when comparing the performance of BBL CHROMagar Listeria and the reference media when USDA/FSIS, FDA/BAM, AOAC, or ISO methods were used for testing raw ground beef, smoked salmon, lettuce, and Brie cheese.

No false-positive colonies were recovered on any sample matrix using BBL CHROMagar Listeria. All presumptively positive colonies were confirmed as *L. monocytogenes* using the reference method identification schemes.

The two lots of naturally contaminated raw ground beef used in the USDA testing were fractionally positive and ranged from 85 to 100% method agreement. In one lot of naturally contaminated raw ground beef tested, BBL CHROMagar Listeria detected three more positives than Modified Oxford ($n = 7$) out of the 10 total positives. This difference was not statistically significant based on a Chi square value of 1.33, but resulted in an 85% method agreement for this lot.

In the testing of raw ground beef and smoked salmon using ISO method 11290, time to recovery differences were observed for ALOA and BBL CHROMagar Listeria. Twenty-seven positive samples were recovered on BBL CHROMagar Listeria after 24 hours of

incubation from the primary (Half Fraser) and secondary (Fraser) broths. Three additional positive samples were detected following 48 hours of incubation. In contrast, three positive samples were detected on ALOA from the primary and secondary broths after 24 hours, with an additional 23 detected following 48 hours of incubation. The 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 media at 24 and 48 hours.

To evaluate the ability of BBL CHROMagar Listeria to detect a range of different strains of *L. monocytogenes*, 54 isolates of *L. monocytogenes* and three strains of *L. ivanovii* were tested. Of the 57 strains tested, five were ATCC™ strains and 52 were food source isolates. Strains were inoculated into the following broths: Modified University of Vermont (UVM) Broth, Buffered Listeria Enrichment Broth with supplements, AOAC Official Method 993.12 Selective Enrichment Medium with supplements, Half Fraser Broth, and ISO 10560 Selective Enrichment Medium with supplements. Broths were incubated according to the reference method instructions and subcultured to BBL CHROMagar Listeria. Plates were incubated at $35^\circ \pm 2^\circ\text{C}$ for 24 hours. If blue-green colonies with opaque, white halos were not observed at 24 hours, plates were incubated an additional 24 hours. BBL CHROMagar Listeria recovered and produced positive results with all strains of *L. monocytogenes* and *L. ivanovii* for a sensitivity of 100%.

To evaluate the ability of BBL CHROMagar Listeria to differentiate *L. monocytogenes* and *L. ivanovii* from other organisms, 56 non-*L. monocytogenes/L. ivanovii* isolates were tested. The 56 isolates represented 16 bacterial genera and yeast, including 25 isolates of *Listeria* species. Each strain was cultured in Brain Heart Infusion (BHI) Broth at 35°C for 24 hours. After incubation, the BHI Broth tubes were subcultured to BBL CHROMagar Listeria

Table 4. Summary of method comparison testing for BBL™ CHROMagar™ Listeria (CL)

Food matrix	Method	Level	MPN/g	Total samples	Total positive	Reference positive	CL positive	Chi square value ^a	Method agreement, % ^b	
Raw ground beef lot 1	USDA	Naturally contaminated	0.094	20	10	7	10	1.33	85	
Raw ground beef lot 2	USDA	Naturally contaminated	0.43	20	8	8	8	0.00	100	
Raw ground beef	ISO	Low	0.061	20	11	11	10	0.00	96.0	
		Control	<0.003	5	0	0	0			
Smoked salmon	FDA (external lab)	Low	0.023	20	19	19	19	0.00	100	
		Control	<0.003	5	0	0	0			
	FDA (internal lab)	Low	0.15	20	18	18	18	0.00	100	
		Control	<0.003	5	0	0	0			
	ISO (external lab)	Low	0.240	20	17	17	17	0.00	100	
		Control	<0.003	5	0	0	0			
	ISO (internal lab)	Low	0.75	20	13	13	13	0.00	100	
		Control	<0.003	5	0	0	0			
	Lettuce	FDA	Low	0.093	20	17	17	17	0.00	100
			Control	<0.003	5	0	0	0		
ISO		Low	0.093	20	9	9	9	0.00	100	
		Control	<0.003	5	0	0	0			
Brie cheese	AOAC	Low	0.0036	20	9	9	9	0.00	100	
		Control	<0.003	5	0	0	0			
	ISO	Low	0.0043	20	19	19	19	0.00	100	
		Control	<0.003	5	0	0	0			
Total				265	150	147	149			

^a A Chi square value < 3.84 indicates no significance at $p < 0.05$.

^b 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 the USDA method.

plates. Plates were incubated at $35^\circ \pm 2^\circ\text{C}$ for 24 hours. Negative plates were incubated for a total of 48 hours. All 56 isolates failed to produce blue-green colonies with opaque, white halos on BBL CHROMagar Listeria. The overall specificity was 100%.

Three lots were evaluated for consistency and stability by testing replicates over the shelf life of the product. BBL CHROMagar Listeria demonstrated reproducible, consistent results that did not degrade over the product shelf life.

Impact on product performance was evaluated by testing under minor variations from the recommended test method. BBL CHROMagar Listeria was able to detect *L. monocytogenes* over incubation temperatures of $32^\circ\text{--}38^\circ\text{C}$. Incubation at 38°C produced results comparable to those obtained at 35°C over 22–50 hours. At an incubation

temperature of 32°C , detection of positive results was delayed to 48 hours for some strains. The package insert recommends incubating 24-hour negative cultures for a total of 48 hours prior to reporting as negative.

Conclusion

The results of these studies demonstrate that BBL CHROMagar Listeria can be used for the detection and identifica-

tion of *L. monocytogenes* and *L. ivanovii* in raw ground beef, smoked salmon, lettuce, and Brie cheese when USDA/FSIS, FDA/BAM, AOAC, and ISO methods are used. With these foods, BBL CHROMagar Listeria provides results equivalent to or better than the reference media, with no confirmatory biochemical screening tests and a quicker time to detection for samples positive for *L. monocytogenes* and *L. ivanovii*. ■

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Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding a test kit that has been granted *Performance Tested MethodsSM* status, contact Deborah McKenzie at dmckenzie@aoac.org.

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