



QUALITY CONTROL PROCEDURES

*See footnote below

I INTRODUCTION

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the cultures listed below.
 - Streak inoculate with 10^3 - 10^4 CFUs of *S. Typhimurium* and *S. Typhi* and 10^4 - 10^5 CFUs of all other organisms.
 - Incubate plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
 - Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 18–24 h for amount of growth, and up to 48 h for color formation of *Salmonella*.
- Expected Results

Organisms	ATCC™	Recovery	Colony Color
<i>Escherichia coli</i>	25922	Inhibition (partial to complete)	Blue to blue-green
* <i>Citrobacter freundii</i>	8090	Fair to heavy growth	Light blue-green to blue-green
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi	19430	Fair to heavy growth	Mauve
* <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	Fair to heavy growth	Light mauve to mauve
* <i>Staphylococcus aureus</i>	25923	Inhibition (partial to complete)	Cream

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification 7.6 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates aerobically at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species from other coliform and non-coliform bacteria in clinical stool samples and a variety of food samples.

BBL CHROMagar Salmonella has been validated by the AOAC™ Research Institute under the Performance Tested MethodsSM program only for the analysis of raw ground beef, raw chicken, raw fish, lettuce and shell eggs. ISO, USDA FSIS and FDA BAM methods were used for method comparison testing.¹⁻³ **BBL CHROMagar Salmonella** was found to be equivalent to the plated media recommended in the ISO, FDA and USDA methods.

U.S. Patent Nos. 5,098,832; 5,194,374

V SUMMARY AND EXPLANATION

Salmonella is ubiquitous in animal populations and is generally isolated from the intestinal tract of animals and humans. It is one of the most prevalent organisms associated with foodborne illnesses, which is often linked to animal origin.⁴ Illnesses caused by *Salmonella* have been associated with poultry, beef, chocolate, dairy and vegetable products.⁵

BBL CHROMagar Salmonella is intended for the isolation and differentiation of *Salmonella* species. The addition of chromogenic substrates in the medium facilitates detection of *Salmonella* species from other flora.

BBL CHROMagar Salmonella 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 Salmonella** prepared plated medium using the **Difco™ CHROMagar Salmonella** dehydrated culture medium formulation.

VI PRINCIPLES OF THE PROCEDURE

Specially selected peptones supply the nutrients. Gram-positive organisms are generally inhibited as a result of the selective medium base. The addition of an antifungal agent prevents the growth of *Candida* species and other antimicrobial agents are used to inhibit the growth of gram-negative, non-glucose fermenting bacteria and *Proteus* species, which could potentially overgrow *Salmonella* colonies. A chromogenic mixture is included in the medium. Due to metabolic differences in the presence of selected chromogens, colonies of *Salmonella* species appear mauve (rose to purple) in color, whereas undesired bacteria are either inhibited, or produce blue-green or colorless colonies.

*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 MethodsSM* CERTIFICATE NUMBER 020502.

VII REAGENTS

BBL CHROMagar Salmonella

Approximate Formula* Per Liter Purified Water

Chromopeptone	22.0 g
Chromogenic Mix	0.34 g
Inhibitory Agents	0.02 g
Agar	15.0 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an 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.

To become familiar with the expected chromogenic (color) reactions produced by *Salmonella*, it is recommended that the user inoculate representative strains commonly observed in their institution. The following strains are suggested: *Salmonella* ser. Typhimurium, ATCC™ 14028; *Salmonella* ser. Dublin, ATCC 15480; *Salmonella* ser. Typhi, ATCC 19430; and *Salmonella enterica* subsp. *arizonae*, ATCC 12323.

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

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

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of sample or specimen collection and handling procedures.¹⁻⁵

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁶⁻⁹ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

IX PROCEDURE

Material Provided: BBL CHROMagar Salmonella

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required for the specific laboratory procedure in use, such as ISO 7218, USDA FSIS MLG, FDA BAM or your specific laboratory procedure.

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

For clinical specimens: As soon as possible after receipt in the laboratory, inoculate the specimen onto a BBL CHROMagar Salmonella plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2 °C in an inverted position (agar-side up) for 24 h. If negative at 24 h, reincubate for an additional 24 h to report final results. Once the colony color develops, exposure to light is permissible. Typical colonies of *Salmonella* should be subjected to confirmatory biochemical or serological testing.

For food samples: Follow sample preparation methodology as outlined in USDA FSIS's *Microbiology Laboratory Guidebook: Isolation and Identification of Salmonella from Meat, Poultry, and Egg Products*, FDA BAM's chapter on *Salmonella*, ISO guidelines or the procedure guidelines appropriate to sample type and geographic location.

Inoculate the incubated enrichment broth sample onto a BBL CHROMagar Salmonella plate. Streak for isolation, incubate plates aerobically at 35 ± 2°C in an inverted position (agar side up) for 24 h. If negative at 24 h, reincubate for an additional 24 h to report final results. Typical colonies of *Salmonella* growing on BBL CHROMagar Salmonella should be subjected to confirmatory testing as outlined in ISO, USDA FSIS and FDA BAM procedures.¹⁻³

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that clinical users refer to pertinent Clinical Laboratory and Standards Institute (CLSI) (formerly known as NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After proper incubation, read plates against a white background. *Salmonella* Typhimurium and other *Salmonella* species will appear as light mauve to mauve-colored colonies, with the exception of *Salmonella enterica* subspecies *arizonae* and other *Salmonella* species positive for lactose and beta-glucosidase. Those isolates will appear as blue-violet or purple colonies. *Citrobacter* and other coliforms will appear as light blue-green to blue-green colored colonies. Some organisms that do not hydrolyze any of the chromogenic compounds may appear as colorless colonies.

XI LIMITATIONS OF THE PROCEDURE

Occasionally strains of *Aeromonas hydrophila*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *P. putida*, *Acinetobacter* species, or *Candida* species may not be completely inhibited and colonies may exhibit light mauve to mauve pigmentation.

Confirmatory tests that use mauve or purple as an indicator color reaction may be difficult to interpret due to the actual colony color.

Rare strains of the following organisms: *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium*, *S. Choleraesuis*, *S. Minnesota*, *S. enterica* subsp. *arizonae*, and *S. Pullorum* may fail to grow or have reduced growth on this medium. This is strain specific and the majority of the strains tested of each of these serovars were recovered.

BBL CHROMagar Salmonella is not designed for the isolation of intestinal pathogens other than *Salmonella*. When testing some samples, a purple discoloration of the medium, without detectable colony growth, may be observed. This should be considered a negative result.

Minimize exposure of **BBL CHROMagar** *Salmonella* to light before and during incubation, as light may destroy the chromogens. Keep plates within the original sleeve wrapping and cardboard box for the entire storage period. Incubation in CO₂ is not recommended.

XII EXPECTED VALUES

The following organisms were isolated during internal and external evaluations of clinical and industrial samples:

<i>Salmonella</i> ser. Abony	<i>Salmonella</i> ser. Gallinarum	<i>Salmonella</i> ser. Oranienburg
<i>Salmonella</i> ser. Adelaide	<i>Salmonella</i> ser. Gaminara	<i>Salmonella</i> ser. Panama
<i>Salmonella</i> ser. Agona	<i>Salmonella</i> ser. Hadar	<i>Salmonella</i> ser. Paratyphi A
<i>Salmonella</i> ser. Anatum	<i>Salmonella</i> ser. Hartford	<i>Salmonella</i> ser. Paratyphi B
<i>Salmonella</i> ser. Bareilly	<i>Salmonella</i> ser. Heidelberg	<i>Salmonella</i> ser. Pomona
<i>Salmonella</i> ser. Berta	<i>Salmonella</i> ser. Illinois	<i>Salmonella</i> ser. Poona
<i>Salmonella</i> ser. Brandenburg	<i>Salmonella</i> ser. Infantis	<i>Salmonella</i> ser. Potsdam
<i>Salmonella</i> ser. California	<i>Salmonella</i> ser. Iverness	<i>Salmonella</i> ser. Pullorum
<i>Salmonella</i> ser. Cerro	<i>Salmonella</i> ser. Javiana	<i>Salmonella</i> ser. Rubislaw
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	<i>Salmonella</i> ser. Johannesburg	<i>Salmonella</i> ser. Schwarzengrund
<i>Salmonella</i> ser. Choleraesuis	<i>Salmonella</i> ser. Kentucky	<i>Salmonella</i> ser. Senftenberg
<i>Salmonella</i> ser. Cubana	<i>Salmonella</i> ser. London	<i>Salmonella</i> ser. St. Paul
<i>Salmonella</i> ser. Derby	<i>Salmonella</i> ser. Mbandaka	<i>Salmonella</i> ser. Thompson
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	<i>Salmonella</i> ser. Michigan	<i>Salmonella</i> ser. Typhi
<i>Salmonella</i> ser. DT 104	<i>Salmonella</i> ser. Minnesota	<i>Salmonella</i> ser. Typhimurium
<i>Salmonella</i> ser. Dublin	<i>Salmonella</i> ser. Montevideo	<i>Salmonella</i> ser. Typhimurium (lactose positive)
<i>Salmonella</i> ser. Enteritidis	<i>Salmonella</i> ser. Muenster	<i>Salmonella</i> ser. Weltevreden
<i>Salmonella</i> ser. Essen	<i>Salmonella</i> ser. Newport	<i>Salmonella</i> 8, (20):-:26

XIII PERFORMANCE CHARACTERISTICS

Clinical Testing:

BBL CHROMagar *Salmonella* was tested at a large diagnostic laboratory. A total of 150 known negative stool specimens and 110 known positive stool specimens were tested on **BBL CHROMagar** *Salmonella* and compared to the performance of XLD and Hektoen Enteric media. The sensitivity and specificity of **BBL CHROMagar** *Salmonella* medium after 18-24 h of incubation were 76% and 99%, respectively; and after 48 h of incubation were 90% and 94%, respectively. The sensitivity and specificity increased to 99% and 97%, respectively, when using Selenite F broth. Comparative sensitivity and specificity results for XLD medium were 71% and 97% at 18-24 h incubation, and 78% and 95% at 48 h incubation; sensitivity and specificity results for Hektoen Enteric medium were 71% and 94% at 18-24 h, and 79% and 93% at 48 h incubation.

Agrifood Testing:

USDA and FDA Methods

BBL CHROMagar *Salmonella* was evaluated for the recovery of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce, and shell eggs in internal and AOAC approved external laboratories. The raw chicken and ground beef were processed according to the USDA FSIS reference methods. The raw fish, lettuce and shell eggs were processed according to the FDA BAM procedures. **BBL CHROMagar** *Salmonella* was compared to the reference method media for the selective recovery of *Salmonella*. A total of 16 positive cultures were obtained from the raw chicken, 17 in the raw ground beef, 18 in the raw fish and lettuce and 11 in the shell egg samples. **BBL CHROMagar** *Salmonella* produced comparable results with the reference methods on all matrices resulting in a method agreement of 100%.

Twenty spiked raw chicken samples were tested according to the USDA FSIS reference method. The chicken was seeded with a low inoculum of 8 CFU/25 g and a high inoculum level of 50 CFU/25 g of sample. **BBL CHROMagar** *Salmonella* recovered 100% (20/20) of the low and high spiked level of *Salmonella*. At inoculum levels less than 1 CFU/25 g, fractional recovery was obtained. Naturally contaminated chicken was tested. Recovery of *Salmonella* was 100% and the Most Probable Number (MPN)/g was 0.23.

One hundred eighteen (118) *Salmonella* isolates of foodborne origin including various serotypes were cultured in Lactose Broth for 24 h and then subcultured to Tetrathionate Broth for 24 h. Tetrathionate Broths were subcultured to **BBL CHROMagar** *Salmonella* and incubated at 35°C. If mauve colonies were not recovered at 24 h, plates were incubated for an additional 24 h. **BBL CHROMagar** *Salmonella* recovered 111 isolates. Four strains were inhibited by Tetrathionate Broth and were not recovered on **BBL CHROMagar** *Salmonella* or on a nonselective control plate. Other isolates of the same serotypes as the four negatives did produce typical colonies so the lack of positive reaction was strain, not serotype, specific. Overall sensitivity for **BBL CHROMagar** *Salmonella* was 94% (111 of 118 isolates).

Sixty-five (65) isolates of non-*Salmonella* were cultured in Brain Heart Infusion Broth at 35°C for 24 h and subcultured to **BBL CHROMagar** *Salmonella* at 35°C for 24 h. Negative plates were incubated for a total of 48 h. Sixty one (61) of the 65 isolates did not exhibit mauve coloration on **BBL CHROMagar** *Salmonella* for a specificity of 94%. The four non-*Salmonella* strains that exhibited mauve coloration were inoculated into Tetrathionate Broth (recommended enrichment broth for USDA and FDA methods). Following 24 h incubation, Tetrathionate Broths were subcultured to **BBL CHROMagar** *Salmonella*. After 48 h of incubation, one strain grew mauve colonies and three strains were inhibited. Based on the use of Tetrathionate Broth enrichment, the overall specificity of **BBL CHROMagar** *Salmonella* in this study was 98%.

ISO Method

BBL CHROMagar *Salmonella* was compared to the reference method media for the selective recovery of *Salmonella* in raw chicken, raw ground beef, raw fish, lettuce, and shell eggs. All matrices were processed according to the ISO culture method. A total of 16 positive cultures were obtained from the raw chicken, 17 in the raw ground beef, 9 in the raw fish, 19 in the lettuce and egg shell samples. **BBL CHROMagar** *Salmonella* produced comparable results with the reference methods on all matrices resulting in a method agreement of 100%.

Twenty (20) samples of raw ground beef, raw fish, lettuce and shell eggs were spiked with a low level inoculum of *Salmonella* and analyzed according to the ISO culture procedure. Twenty (20) samples of naturally contaminated raw chicken were analyzed according to the ISO culture procedure. **BBL CHROMagar** *Salmonella* was added to the battery of reference media for

each food matrix tested. The method agreement of **BBL CHROMagar** Salmonella and the other reference media tested was 100%. The low inoculum levels ranged from 0.0036 to 0.23 MPN/g.

One hundred twenty seven (127) *Salmonella* isolates of foodborne origin were cultured in Buffered Peptone Water for 18 h and then subcultured to Rappaport-Vassiliadis with Soya (RVS) medium for 24 h. RVS broths were subcultured to **BBL CHROMagar** Salmonella and incubated at 35°C for 24 h. If mauve colonies were not recovered at 24 h, plates were incubated for an additional 24 h. **BBL CHROMagar** Salmonella recovered 123 isolates. Recovery of the 4 strains that did not grow on **BBL CHROMagar** Salmonella was poor on the nonselective control media. Overall sensitivity for **BBL CHROMagar** Salmonella was 96.8% (123 of 127 isolates).¹⁰

XIV AVAILABILITY

Cat. No.	Description
214983	BBL™ CHROMagar™ Salmonella, Pkg. of 20 plates

XV REFERENCES

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