INTENDED USE

BBL™ CHROMagar™ Salmonella is a selective and differential medium for the isolation and presumptive identification of Salmonella species from clinical stool specimens.

BBL CHROMagar Salmonella has also 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.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

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.

In BBL CHROMagar Salmonella 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.

REAGENTS

Formula** Per Liter Purified Water

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromopeptide</td>
<td>22.0 g</td>
</tr>
<tr>
<td>Chromogenic Mix</td>
<td>0.34 g</td>
</tr>
<tr>
<td>Inhibitory Agents</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
</tbody>
</table>

pH 7.7 +/-0.2

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

*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.
PRECAUTIONS

For professional use only. 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 the bottom of the plate during incubation.

Protect from light during drying. See STORAGE AND SHELF LIFE.

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.

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.

Consult GENERAL INSTRUCTIONS FOR USE document for details and for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

Product Deterioration

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

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:

<table>
<thead>
<tr>
<th>Strains</th>
<th>Growth Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> Typhimurium ATCC 14028</td>
<td>Fair to heavy growth of light mauve to mauve (rose to purple) colored colonies</td>
</tr>
<tr>
<td><em>Salmonella</em> Enteritidis ATCC 13076</td>
<td>Growth; mauve (=rose to purple) colonies</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> ATCC 8090</td>
<td>Fair to heavy growth of light blue-green to blue-green colored colonies</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>Inhibition partial to complete; colonies blue-green</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 33495</td>
<td>Inhibition partial; colonies blue-green</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 43071</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>Inhibition partial to complete; colonies small, blue-green</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td>Appearance of uninoculated medium</td>
<td>Colorless to light amber, transparent</td>
</tr>
</tbody>
</table>

Note: 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.

PROCEDURE

Materials Provided

BBL CHROMagar Salmonella (90 mm Stacker™ plates). Microbiologically controlled.
**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.

**Specimen Collection**
Refer to appropriate texts for details of sample or specimen collection and handling procedures.\(^1\)\(^-\)\(^5\)

**Specimen Types**
This medium can be used with clinical stool specimens and a variety of food samples (raw ground beef, raw chicken, raw fish, lettuce and shell eggs).

**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. Depending on local procedures, inoculation of additional media for the isolation of *Salmonella* and for the detection of other enteric pathogens may be mandatory. 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 of **BBL CHROMagar** Salmonella to light is permissible. Typical colonies of *Salmonella* should be subjected to confirmatory biochemical or serological testing.

**BBL CHROMagar** Salmonella may also be used for subculturing from pre-enrichment media such as Selenite Broth or Tetrathionate Broth.

*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.\(^1\)\(^-\)\(^3\)

**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.

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**
**BBL CHROMagar** Salmonella is a selective and differential medium for the isolation and presumptive identification of *Salmonella* species from clinical stool specimens and a variety of food samples.

**Expected Results**
*Salmonella* species will appear as light mauve to mauve (=rose to purple) colored colonies on this medium, with the exception of *Salmonella* species positive for lactose and beta-glucosidase. Those isolates will appear as blue-violet or purple colonies.
The following organisms were isolated on BBL CHROMagar Salmonella during internal and external evaluations of clinical and industrial samples:

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

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 were 90% and 94% after 48 h of incubation, respectively. The sensitivity and specificity increased to 99% and 97%, respectively, when subculturing from 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-Vassiliades 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).

**Limitations of the Procedure**

Occasionally strains of *Aeromonas hydrophila*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *P. putida*, *Stenotrophomonas maltophilia*, *Acinobacter* 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.

**REFERENCES**


**PACKAGING/AVAILABILITY**

**BBL CHROMagar Salmonella**

**REF** 254104 Ready-to-use Plated Media, cpu 20

**FURTHER INFORMATION**

For further information please contact your local BD representative.

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*Becton Dickinson GmbH*

Tullastrasse 8 – 12

D-69126 Heidelberg/Germany

Phone: +49-62 21-30 50  Fax: +49-62 21-30 52 16

Reception_Germany@europe.bd.com

http://www.bd.com

http://www.bd.com/europe/regulatory/

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