**INTENDED USE**

BBL CHROMagar Salmonella is a selective differential medium for the isolation and presumptive identification of Salmonella, and XLD Agar (Xylose Lysine Desoxycholate Agar) is a moderately selective and differential medium for the isolation of Salmonella and Shigella. The combination of both media in a biplate allows simultaneous detection of Shigella and Salmonella.

**PRINCIPLES AND EXPLANATION OF THE PROCEDURE**

Microbiological method.

Salmonella is one of the leading pathogens in producing food-borne gastroenteritis. Therefore, many different media have been developed for the isolation from feces, foods, and other materials.\(^1\)

BBL CHROMagar Salmonella contains proprietary chromogenic substrates to stain Salmonella colonies in rose-violet (=mauve) to blue-violet. Additional chromogenic substrates stain most non-Salmonella organisms in blue-green. Species not reacting with any of the chromogenic substrates will appear in their natural colony color (colorless to grey). Due to the inhibitory agents included in the medium, many non-Salmonella bacteria are inhibited.

In BBL CHROMagar Salmonella specially selected peptones supply the nutrients. Gram-positive organisms and fungi are generally inhibited as a result of the selective medium base. Other inhibitors are used to reduce 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, violet or purple), whereas undesired bacteria are either inhibited, or produce blue-green or colorless colonies. Since the appearance of mauve colonies is very specific for Salmonella, biochemical confirmation tests are usually unnecessary when using BBL CHROMagar Salmonella. If a sufficient number of isolated mauve colonies is present, slide agglutination tests necessary to confirm the strain as Salmonella may be performed directly from the isolation plate without further subcultures (see PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).

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.

XLD Agar is a moderately selective and differential medium. It contains yeast extract as a source of nutrients and vitamins. It utilizes sodium desoxycholate as the selective agent and, therefore, is inhibitory to gram-positive micro-organisms. Xylose is incorporated into the medium since it is fermented by practically all Enterobacteriaceae except for the shigellae and this property enables the differentiation of Shigella species. Lysine is included to enable the Salmonella group to be differentiated from the non pathogens since without lysine, salmonellae rapidly would ferment the xylose and be indistinguishable from nonpathogenic species. After the salmonellae exhaust the supply of xylose, the lysine is attacked via the enzyme lysine decarboxylase, with reversion to an alkaline pH which mimics the Shigella reaction. To prevent similar reversion by lysine positive coliforms, lactose and sucrose are added to produce acid in excess.\(^2\)\(^6\)

Additionally, an H\(_2\)S indicator system, consisting of sodium thiosulfate and ferric ammonium citrate, is included for the visualization of the hydrogen sulfide produced, resulting in the
formation of colonies with black centers. The non pathogenic H$_2$S-producers do not
decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of
the colonies which takes place only at neutral or alcaline pH.

The presence of BBL CHROMagar Salmonella and XLD Agar in a biplate combines the highly
selective chromogenic medium which allows rapid presumptive identification of Salmonella by
colony color with the moderate selectivity of XLD Agar which increases the chance of recovery
when the bacterial population is low and provides an indication of the presence of Shigella in the
specimen. Additionally, the biplate satisfies the requirement to use two different media for
isolation of Salmonella.  

REAGENTS
BBL CHROMagar Salmonella / XLD Agar / (Biplate)

<table>
<thead>
<tr>
<th>Formula* Per Liter Purified Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL CHROMagar Salmonella</td>
</tr>
<tr>
<td>Chromopeptide</td>
</tr>
<tr>
<td>Chromogenic Mix</td>
</tr>
<tr>
<td>Inhibitory Agents</td>
</tr>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>pH 7.7 +/- 0.2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>XLD Agar</td>
</tr>
<tr>
<td>Xylose</td>
</tr>
<tr>
<td>L-Lysine</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Yeast Extract</td>
</tr>
<tr>
<td>Phenol Red</td>
</tr>
<tr>
<td>Sodium Desoxycholate</td>
</tr>
<tr>
<td>Sodium Thiosulfate</td>
</tr>
<tr>
<td>Ferric Ammonium Citrate</td>
</tr>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>pH 7.4 +/- 0.2</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented to meet the performance criteria.

PRECAUTIONS

For professional use only.

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

Before using this medium for the first time, we recommend to train the typical colony
appearance with defined strains, e.g., by using the strains mentioned under USER QUALITY
CONTROL.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus,
may be present in clinical specimens. Standard Precautions and institutional guidelines should
be followed in handling all items contaminated with blood and other body fluids.
Consult GENERAL INSTRUCTIONS FOR USE document 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 and cardboard
box for the entire storage period. 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.

Minimize exposure to light before and during incubation, since light may destroy the
chromogens included in BBL CHROMagar Salmonella.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see GENERAL
INSTRUCTIONS FOR USE document). Incubate plates at 35 ± 2° C in an aerobic atmosphere.
Examine plates after 24 hours of incubation.
### Test strains

<table>
<thead>
<tr>
<th>Test strains</th>
<th>BBL CHROMagar Salmonella</th>
<th>XLD Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC™ 14028</td>
<td>Growth; mauve (=rose-violet) to violet colonies</td>
<td>Growth; black colonies or red colonies with black centers</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076</td>
<td>Growth; mauve (=rose-violet) to violet colonies</td>
<td>Growth; black colonies or red colonies with black centers</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> ATCC 12022</td>
<td>Growth; colorless colonies</td>
<td>Growth; red colonies</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>Inhibition partial to complete; colonies blue-green</td>
<td>Inhibition partial to complete; yellow colonies</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> ATCC 43071</td>
<td>Inhibition partial to complete</td>
<td>Growth; colonies rose to red; may have black centers; swarming inhibited</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 33495</td>
<td>Inhibition partial; colonies blue-green</td>
<td>Growth; yellow colonies</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Colorless to light amber</td>
<td>Red</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 / XLD Agar (Biplate), provided in 90 mm Stacker™ biplates. 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.

**Specimen Types**

The media included in this biplate are used for the detection of *Salmonella* and *Shigella* from stool specimens or rectal swabs of patients suspected to have a bacterial enteric infection. Other specimens suspected to contain *Salmonella* or *Shigella* may also be used. It may also be used as a medium for subculturing from pre-enrichment broth for *Salmonella* (Selenite F Broth).

**Test Procedure**

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. With a 10 µl loop or the swab, first inoculate a small area of XLD Agar, and afterwards a small area of BBL CHROMagar Salmonella. Using a fresh loop for each of the media, streak for isolation from the inoculated areas. A less selective medium such as BD MacConkey II Agar should also be included to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Incubate the inoculated plates aerobically at 35 ± 2°C for 24 hours. If negative, re-incubate for additional 24 hours and read for a second time.

**Results**

The presence of mauve colonies on BBL CHROMagar Salmonella together with black colonies or red colonies with black centers on XLD Agar is highly predictive for *Salmonella*, with the exception of *Salmonella enterica* subspecies *arizonae* and other *Salmonella* species positive for lactose fermentation and beta-glucosidase. Those isolates on BBL CHROMagar Salmonella will appear as blue-violet or purple colonies. While a biochemical identification of mauve colonies is usually unnecessary, standard serological tests such as slide agglutination must be used for a complete diagnosis (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE). A standard oxidase test (performed on filter paper with growth from BBL CHROMagar Salmonella Medium) is recommended for non-agglutinating, mauve colonies to determine the presence of oxidase positive non-fermenters or *Aeromonas hydrophila* (=oxidase positive) which occasionally produce rose to mauve colonies. When performing an oxidase test
from mauve colonies, the color of a negative test is mauve to violet, while the color of a positive test is dark blue to black. It is recommended to include a *Salmonella* strain as a negative control.

Presence of colorless or blue-green colonies on **BBL CHROMagar Salmonella** must not be taken as an indication for the presence of *Shigella*. Perform biochemical and serological tests for *Shigella* from growth on **XLD Agar** only. On this medium, *Shigella* strains will usually produce red, rarely yellowish colonies.

Typical appearance of the organisms is as follows:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>BBL CHROMagar Salmonella</th>
<th>XLD Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli, <em>Citrobacter</em></td>
<td>Inhibited or blue-green colonies with or without mauve halos</td>
<td>Large, flat, yellow. Some strains may be inhibited.</td>
</tr>
<tr>
<td><em>Enterobacter</em>/</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>Partially inhibited; blue-green to blue colonies with or without mauve halos</td>
<td>Mucoid, yellow</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>Inhibition partial to complete</td>
<td>Red to yellow. Most strains have black centers.</td>
</tr>
<tr>
<td><em>Salmonella</em>, H$_2$S-positive</td>
<td>Growth; mauve (=rose-violet) to violet colonies*</td>
<td>Black or red with black centers.</td>
</tr>
<tr>
<td><em>Salmonella</em>, H$_2$S-negative</td>
<td>Partially to completely inhibited; colorless or (rarely) blue-green colonies</td>
<td>Red</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Inhibition partial to complete</td>
<td>Red</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila,</em></td>
<td>Inhibition partial to complete; may rarely produce rose to mauve colonies; oxidase positive (S. maltophilia may be weakly positive or negative)*</td>
<td>Yellow or pink</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See Limitations of the Procedure*

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**

**BBL CHROMagar Salmonella / XLD Agar (Biplate)** is used for the primary isolation of *Salmonella* and *Shigella* from fecal specimens or from enrichments for *Salmonella* (Selenite Broth). Additionally, **BBL CHROMagar Salmonella** allows the presumptive identification of *Salmonella*. Additional tests are needed for confirmation.

**Performance Results**

The following *Salmonella* strains have been isolated on **BBL CHROMagar Salmonella** during internal and external evaluations:

- *Salmonella* 8, (20):-:26
- *Salmonella* enterica subsp. *arizonae*
- *Salmonella* enterica subsp. *diarizonae*
- *Salmonella* Abony
- *Salmonella* Adelaide
- *Salmonella* Agona
- *Salmonella* Anatum
- *Salmonella* Bareilly
- *Salmonella* Berta
- *Salmonella* Brandenburg
- *Salmonella* California
- *Salmonella* Cerro
- *Salmonella* Choleraesuis
- *Salmonella* Cubana
- *Salmonella* Derby
- *Salmonella* DT 104
- *Salmonella* Dublin
- *Salmonella* Enteritidis
- *Salmonella* Essen
- *Salmonella* Gallinarum
- *Salmonella* Gaminara
- *Salmonella* Hadar
- *Salmonella* Hartford
- *Salmonella* Heidelberg
- *Salmonella* Illinois
- *Salmonella* Infantis
- *Salmonella* Iverness
- *Salmonella* Javiana
- *Salmonella* Johannesburg
- *Salmonella* Kentucky
- *Salmonella* London
- *Salmonella* Mbandaka
- *Salmonella* Michigan
- *Salmonella* Minnesota
- *Salmonella* Montevideo
- *Salmonella* Muenster
- *Salmonella* Newport
- *Salmonella* Oranienburg
- *Salmonella* Panama
- *Salmonella* Paratyphi A
- *Salmonella* Paratyphi B
- *Salmonella* Pomonae
- *Salmonella* Poona
- *Salmonella* Potsdam
- *Salmonella* Pullorum
- *Salmonella* Rubislaw
- *Salmonella* Schwarzengrund
- *Salmonella* Senftenberg
- *Salmonella* St. Paul
- *Salmonella* Thompson
- *Salmonella* Typhi
- *Salmonella* Typhimurium
- *Salmonella* Typhimurium (lactose positive)
- *Salmonella* Weltevreden
In an external performance evaluation with 110 known positive and 150 known negative clinical stool specimens, **BBL CHROMagar Salmonella** (=BCAS) was compared to XLD Agar (=XLD) and Hektoen Enteric Agar (=HEA). Sensitivities after 20 hours of incubation were 76, 71, and 71%, and specificities were 99, 97, and 94% for BCAS, XLD, and HEA, respectively. After 42 to 45 hours, sensitivities were 90, 78, and 79% and specificities were 94, 95, and 93% for BCAS, XLD, and HEA, respectively. Positive and negative specimens were also enriched in Selenite F Broth and were subcultured onto Salmonella Shigella Agar (= SSA) and BCAS. The sensitivities in this test were 98 and 99%, and specificities were 81 and 99% for SSA and BCAS, respectively.

### Limitations of the Procedure

**BBL CHROMagar Salmonella**

Occasionally, strains of *Aeromonas hydrophila*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *P. putida*, *Stenotrophomonas maltophilia*, *Acinetobacter* species, or *Candida* species may not be completely inhibited and colonies may exhibit light mauve to mauve pigmentation. Rare strains of *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium*, *S. Choleraesuis*, *S. Minnesota*, *S. enterica* subsp. *arizonae*, *S. Gallinarum* and *S. Pullorum* may fail to grow or have reduced growth. This is strain specific and the majority of the strains tested of each of these serovars

<table>
<thead>
<tr>
<th>Typical growth on BBL CHROMagar Salmonella and XLD Agar</th>
<th>Typical growth on XLD Agar only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Abony</td>
<td>Shigella boydii</td>
</tr>
<tr>
<td>Salmonella Augustenborg</td>
<td>Shigella dysenteriae</td>
</tr>
<tr>
<td>Salmonella Bovismorificans</td>
<td>Shigella flexneri</td>
</tr>
<tr>
<td>Salmonella Gallinarum**</td>
<td>Shigella sonnei</td>
</tr>
<tr>
<td>Salmonella Chinchol</td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica subsp. arizonae</td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica subsp. Hadar</td>
<td></td>
</tr>
<tr>
<td>Salmonella Heidelberg</td>
<td></td>
</tr>
<tr>
<td>Salmonella Schottmuelleni (Paratyphi B)</td>
<td></td>
</tr>
<tr>
<td>Salmonella Senftenburg</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td></td>
</tr>
<tr>
<td>Salmonella Virchow</td>
<td></td>
</tr>
</tbody>
</table>

* one strain needed 2 days incubation for full recovery and colony pigmentation on one or both media
** weak growth after 2 days incubation on CHROMagar Salmonella, no or weak growth on XLD Agar

On **BBL CHROMagar Salmonella**, most *Salmonella* strains yielded light to dark mauve (violet) colonies; *Salmonella enterica* subsp. *arizonae* strains yielded violet colonies with a blue-green hue. *Salmonella* Gallinarum usually needed 2 days for acceptable growth and coloration and in some tests was not recovered from one or both media of this biplate; this organism is very rarely isolated from human specimens. On **BBL CHROMagar Salmonella** all non-*Salmonella* and non-*Shigella* test strains except *Aeromonas hydrophila*, *Acinetobacter baumannii* and *Candida albicans* were either inhibited or yielded blue, blue-green or colorless colonies. *A. hydrophila* and *A. baumannii* occasionally produced weak growth of rose colonies when the medium was challenged with \( \geq 10^5 \) CFU per plate. *C. albicans* occasionally produced white colonies after 24 h incubation but became pale rose to rose after 48 h. Growth of all *Salmonella* (except *S. Gallinarum*) and *Shigella* test organisms on XLD Agar was typical and was not affected by the adjacent **BBL CHROMagar Salmonella** medium.

Additionally, the following *Salmonella* strains were subjected to slide agglutination tests with polyvalent *Difco™* O-antisera (groups A, B, D, E1 –E4, L and C1, C2, F, G, H), using 24 h growth from **BBL CHROMagar Salmonella** and from Columbia Agar with 5% Sheep Blood: *Salmonella* Abony, *S. Augustenborg*, *S. Bovismorificans*, *S. Enteritidis*, *S. Gallinarum*, *S. Glostrup*, *S. Hadar*, *S. Heidelberg*, *S. Oritamerin*, *S. Panama*, *S. Saintpaul*, *S. Senftenberg*, *S. Typhimurium* S. Virchow. Agglutination controls with saline were included. All Salmonella strains from both media produced agglutination with the appropriate antisera. Nonspecific agglutination was not found. When pale rose to pale mauve growth of *Aeromonas hydrophila* and *Candida albicans* from **BBL CHROMagar Salmonella** (after 48 h incubation) was tested as described above, agglutination did not occur.

### Limitations of the Procedure

**BBL CHROMagar Salmonella**:

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

*Rare strains of *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium*, *S. Choleraesuis*, *S. Minnesota*, *S. enterica* subsp. *arizonae*, *S. Gallinarum* and *S. Pullorum* may fail to grow or have reduced growth. This is strain specific and the majority of the strains tested of each of these serovars
were recovered. Therefore, the use of MacConkey Agar as a less selective medium in addition to this biplate is recommended. For an optimal detection and color development of *Salmonella* Typhi, 42 to 48 hours of incubation are necessary.

Confirmatory tests that use mauve or purple as an indicator color reaction may be difficult to interpret due to the actual colony color. When testing some stool specimens, a purple discoloration of the BBL CHROMagar *Salmonella* medium, without detectable colony growth, may be observed. This should be considered a negative result. Tests for *Shigella* must not be performed from BBL CHROMagar *Salmonella* Agar included in this biplate.

**XLD Agar:**
*Proteus* may mimic *Salmonella* on this medium. Confirmatory tests are needed. Rare *Shigella* strains produce only weak growth on XLD Agar. Therefore, the use of MacConkey Agar as a less selective medium in addition to this biplate is recommended.

For a final diagnosis, appropriate confirmatory tests (e.g., slide agglutination tests) are needed.

These media are not designed for the isolation of intestinal pathogens other than *Salmonella* and *Shigella*.

**REFERENCES**
8. Data on file. BD Diagnostic Systems

**PACKAGING/AVAILABILITY**

BBL CHROMagar *Salmonella* / XLD Agar (Biplate)

**REF** 257372 Ready-to-use Plated Media, 20 plates

**FURTHER INFORMATION**

For further information please contact your local BD representative.

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