BD BBL™ CHROMagar™ Salmonella* / XLD Agar (Biplate)

* U.S. Patent # 5,098,832, 5,194,374

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_2S 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_2S-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*.[1,7]

REAGENTS

BBL CHROMagar Salmonella / XLD Agar / (Biplate)

Formula* Per Liter Purified Water

BBL CHROMagar Salmonella	
Chromopeptone	22.0 g
Chromogenic Mix	0.34 g
Inhibitory Agents	0.02 g
Agar	15.0 g
pH 7.7 +/-0.2	

XLD Agar	
Xylose	3.5 g
L-Lysine	5.0
Lactose	7.5
Sucrose	7.5
Sodium Chloride	5.0
Yeast Extract	3.0
Phenol Red	0.08
Sodium Desoxycholate	2.5
Sodium Thiosulfate	6.8
Ferric Ammonium Citrate	0.8
Agar	13.5
pH 7.4 +/- 0.2	

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

PRECAUTIONS

IVD . 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	BBL CHROMagar Salmonella	XLD Agar
Salmonella Typhimurium ATCC™ 14028	Growth; mauve (=rose-violet) to violet colonies	Growth; black colonies or red colonies with black centers
Salmonella Enteritidis ATCC 13076	Growth; mauve (=rose-violet) to violet colonies	Growth; black colonies or red colonies with black centers
Shigella flexneri ATCC 12022	Growth; colorless colonies	Growth; red colonies
Escherichia coli ATCC 25922	Inhibition partial to complete; colonies blue-green	Inhibition partial to complete; yellow colonies
Proteus mirabilis ATCC 43071	Inhibition partial to complete	Growth; colonies rose to red; may have black centers; swarming inhibited
Klebsiella pneumoniae ATCC 33495	Inhibition partial; colonies blue-green	Growth; yellow colonies
Uninoculated	Colorless to light amber	Red

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:

Organisms	BBL CHROMagar Salmonella	XLD Agar
E. coli, Citrobacter	Inhibited or blue-green colonies with or without mauve halos	Large, flat, yellow. Some strains may be inhibited.
Enterobacter/ Klebsiella	Partially inhibited; blue-green to blue colonies with or without mauve halos	Mucoid, yellow
Proteus	Inhibition partial to complete	Red to yellow. Most strains have black centers.
Salmonella, H_2S-positive	Growth; mauve (=rose-violet) to violet colonies*	Black or red with black centers
Salmonella, H_2S-negative		Red
Shigella	Partially to completely inhibited; colorless or (rarely) blue-green colonies	Red
Pseudomonas aeruginosa	Inhibition partial to complete	Red
Aeromonas hydrophila, Stenotrophomonas maltophilia	Inhibition partial to complete; may rarely produce rose to mauve colonies; oxidase positive (*S. maltophilia* may be weakly positive or negative)*	Yellow or pink
Gram-positive bacteria	Inhibition partial to complete	Inhibition partial to complete

*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 [8]

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 Pomona
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.

On **BBL CHROMagar Salmonella / XLD Agar (Biplate)**, the following *Salmonella* serovars and *Shigella* species were tested and recovered in internal evaluations:[8]

Typical growth on **BBL CHROMagar Salmonella** and **XLD Agar** :

Salmonella Abony
Salmonella Augustenborg
Salmonella Bovismorbificans
Salmonella Chincol
Salmonella enterica subsp. a*rizonae* *
Salmonella Enteritidis
Salmonella Gallinarum**
Salmonella Glostrup
Salmonella Group B
Salmonella Hadar
Salmonella Heidelberg
Salmonella Ohio
Salmonella Oranienburg
Salmonella Oritamerin
Salmonella Panama
Salmonella Saintpaul
Salmonella Schottmuelleri (Paratyphi B)
Salmonella Senftenberg
Salmonella Typhi*
Salmonella Typhimurium
Salmonella Virchow

Typical growth on **XLD Agar** only:

Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei

* 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. a*rizonae* 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 >/= 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.* Bovismorbificans, *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 mimick *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

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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

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