BD BBL™ CHROMagar™ ESBL (Biplate)

INTENDED USE

BBL CHROMagar ESBL (Biplate) is a selective chromogenic screening medium for the isolation of *Enterobacteriaceae* and certain other Gram negative rods producing extended-spectrum beta lactamases (ESBL). Appropriate specimens include rectal swabs and a variety of other clinical specimens (see **Specimen Types**). Also, the medium allows for the identification of *E. coli* without further confirmatory tests and for the detection of *the Klebsiella-Enterobacter-Citrobacter-Serratia* and *Proteus-Morganella-Providencia* groups of organisms if the isolates are resistant to the antibiotics included in the medium. Isolates obtained on this medium must be confirmed to be ESBL producers by additional tests.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Resistance to broad-spectrum beta-lactams, including third generation cephalosporins is mediated by a variety of resistance mechanisms. Among those, the plasmid-mediated resistance due to extended-spectrum beta-lactamases (ESBL) is the most important mechanism since it is epidemically spread in intensive-care units and other hospital environments. Typically, strains producing ESBL are sensitive to beta lactamase inhibitors (such as clavulanic acid), cephamycins (e.g., cefoxitin), and carbapenems.[1,5] More recently, strains expressing resistance to carbapenems (carbapenemase producers) have been reported that (with the exception of a few OXA-48 producers) show resistance against third generation cephalosporins, too.[2]
These types of beta-lactamase enzymes have been found in *Klebsiella*, *Escherichia coli* and, although more rarely, in other genera of the *Enterobacteriaceae.*

BBL CHROMagar ESBL (Biplate) is based upon **BBL CHROMagar Orientation** which 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 Orientation** prepared plated medium. In **BBL CHROMagar Orientation Medium**, specially selected peptones supply the nutrients. The chromogen mix consists of artificial substrates (chromogens) which release differently colored compounds upon degradation by specific microbial enzymes, thus assuring the direct differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests. By the development of different colors, the chromogenic media provided in **BBL CHROMagar ESBL (Biplate)** allow for the easy detection of mixed cultures of Gram negatives and the identification of *E. coli* (rose to mauve) without further confirmatory tests and for the detection of the *Klebsiella-Enterobacter-Citrobacter-Serratia* (blue-green to blue) and *Proteus-Morganella-Providencia* (colorless to tan with brown halos extending into the medium) and other Gram negative rods (appearing in their natural color) if the isolates are resistant to the extended spectrum cephalosporins included in media.
BBL CHROMagar ESBL (Biplate) consists of two media, filled in a biplate. Each of the two media contains a different third generation cephalosporin in an appropriate concentration to allow detection of resistance, together with other selective agents to inhibit the accompanying flora present in the specimen. Medium 2 has been supplemented with titanium dioxide to allow easy visual differentiation of the two media. Both media must be inoculated with the same specimen or isolate. Gram negative bacteria such as *Enterobacteriaceae* and certain nonfermenters, if they are resistant to the antimicrobials included, will produce growth on the medium.
With traditional methods, specimens suspected to contain ESBL producers must be plated first on standard isolation media to obtain pure cultures. After incubation, they must be tested for susceptibility. The process of isolation and susceptibility testing takes at least 48 hours. Since

only a relatively small percentage of specimens contains ESBL producers, this is time-consuming and costly.
Using **BBL CHROMagar ESBL (Biplate)**, the specimen is streaked on both media of the plate. After an overnight incubation for 18 to 28 hours (ideal is 20-22 hours), growth of an isolate on one or both media indicates the presence of a potential ESBL producer. Confirmation by susceptibility tests or molecular methods is necessary.
As compared to non-selective isolation followed by susceptibility testing, the use of this product reduces the workload and accelerates the time to detection of ESBL.

REAGENTS

BBL CHROMagar ESBL (Biplate)
Formula* Per Liter Purified Water

Medium 1 (clear)		Medium 2 (turbid)	
Chromopeptone	16.1 g	Chromopeptone	16.1 g
Chromogen Mix	1.3	Chromogen Mix	1.3
Selective agents	0.24	Selective agents	0.24
Agar	15.0	Titanium oxide (insoluble)	0.35
		Agar	15.0
pH 6.8 +/- 0.2			

*Adjusted and/or supplemented as required to meet 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.
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 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 in the dark. **Minimize exposure to light before and during incubation, since light may destroy the chromogens.**

USER QUALITY CONTROL

Inoculate representative samples with the following strains on each medium (for details, see **Specimen Types** and **Test Procedure**). Incubate the plates, preferably in an inverted position, at 35 to 37° C aerobically for 20-22 hours.

Strains	**Growth Results (both media)**
E. coli DSM 22314 (ESBL producer)	Medium 1 (clear): growth moderate to excellent; colonies rose to mauve. Medium 2 (turbid): no growth to moderate growth; colonies rose to mauve
Klebsiella pneumoniae ATCC™ 700603 (ESBL producer)	Both media: growth moderate to excellent; blue to blue-green colonies
Escherichia coli ATCC 25922 (non-ESBL producer)	Both media: inhibition complete
Enterococcus faecalis ATCC 29212	Both media: inhibition complete
Candida albicans ATCC 60193	Both media: inhibition complete

Uninoculated	Medium 1 (clear): transparent, colorless to very light amber (may contain up to a moderate amount of small particles) Medium 2 (turbid): opaque, white to cream

PROCEDURE

Materials Provided

BBL CHROMagar ESBL (Biplate) (90 mm **Stacker™** biplates). Microbiologically controlled.

Materials Not Provided but Required

Ancillary culture media, reagents and laboratory equipment.

Specimen Types

This product is mainly used in the detection of colonization by ESBL producing strains to aid in the prevention and control of ESBL infections in healthcare settings, especially from intensive care units. It is primarily used with rectal swabs but can be used with clinical specimens from other body sites (such as nasal, wound, throat, urethral, and groin swabs), suspected to contain extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* or other Gram negative aerobically growing rods with high resistance to extended spectrum beta lactams. Use of transport devices approved for the collection of microbiological clinical specimens is recommended. Follow the transport device manufacturer's recommended procedures.
The user may also refer to the appropriate references for details of specimen collection and handling procedures.[3,4]
It may also be used for subculturing potential ESBL producing strains from other media. Direct inoculation with colonies is not recommended. To avoid over-inoculation, the colonies should be suspended in saline first (see **Test Procedure**), and a loopful should be streaked on each medium.

Test Procedure

CHROMagar ESBL must be inoculated directly from the swab, without pre-enrichment, or from an isolated colony suspended in saline to match approximately 0.5 McFarland turbidity. Direct inoculation from isolated colonies is not recommended because the high level of inoculum may rarely cause false positive results.
Inoculate the specimen with a swab or loop onto <u>both</u> media of a **BBL CHROMagar ESBL (Biplate)** plate and streak for isolation, using a loop. The following procedure for inoculation must be strictly applied to obtain isolated colonies with their typical appearance. <u>Insufficient inoculation or inoculation of the whole media surfaces with swabs only (without using loops for isolation streaking) may lead to wrong results or may render the plate unreadable. Do not inoculate more than one specimen per plate. Both sides of this biplate must be inoculated with the same specimen.</u>

Inoculation and incubation procedure:

1. Dab the specimen swab on a small area of the first medium of **BBL CHROMagar ESBL (Biplate)**: Do not over-inoculate! Remove the swab from the medium just inoculated.
2. <u>Slightly turn the swab</u> and inoculate the second medium of **BBL CHROMagar ESBL (Biplate)**: dab the swab onto the first streak area of the second medium. Do not over-inoculate!

Note that the sequence of inoculation does not have an influence on the outcome.

3. Return the swab to its specimen tube.
4. <u>With loops</u>, complete the streaking on the plates. Streak for isolation! First complete the first streak areas and streak the second and third areas of both media. It is recommended to use a fresh loop for each side of the test product.
5. Incubate aerobically at 35 to 37° C for 18 to 28 hours (ideal is 20-22 hours), preferably in an inverted position (medium side up). <u>Do not incubate longer and do not incubate in an atmosphere enriched with carbon dioxide.</u> **Avoid exposure to light during incubation as this might destroy the chromogens.** Once the colors of the colonies have developed, exposure to light is permissible.

6. Read plates as described in **Results and Interpretation**.

Depending on the type and purpose of the specimen, other media must also be inoculated to allow for a complete detection of all pathogens contained. Such media include at least a nonselective blood agar plate.

Results and Interpretation

After incubation, specimens containing isolates resistant to the inhibitors included in the media will grow. Although most ESBL producing strains will grow on both media of the biplate, there exist strains with an in-vitro susceptibility to either of the antimicrobials which, therefore, will grow only on one of the media. The plates should show isolated colonies in the areas where the inoculum was diluted appropriately. Appropriate susceptibility tests or molecular methods must be performed to confirm the presence of ESBL producing isolates.

Absence of growth on **both** media indicates that the specimen does not contain strains with resistance to the antimicrobials included in the media.

Note that discoloration of the media without visible colonies (which may occur if the media have been over-inoculated with stool specimens or with excessively high bacterial loads) is considered a negative result (see also **Limitations of the Procedure**).

Differentiation and/or identification of the isolate(s) by colony color and appearance

Rose to pink (mauve) colonies: *Escherichia coli*; an optional indole test using **BD BBL DMACA Indole Reagent Droppers** (cat. no. 261187) may be performed on filter paper for confirmation of *E. coli* (indole positive). Do not apply the indole reagent to the medium surface!

Note: certain *Citrobacter* strains, such as *Citrobacter braakii* have been found to produce violet to lilac colonies on **BBL CHROMagar Orientation** and, if they are resistant to the antibiotics included, on **BBL CHROMagar ESBL (Biplate)**. Biochemical identification is recommended for such strains.

Blue to blue-green colonies which may or may not be surrounded by a rose to mauve zone: *Klebsiella, Enterobacter, Serratia, Citrobacter* or others. Further tests are necessary for identification. For details, consult the Instructions for Use of **BBL CHROMagar Orientation** (see: http://www.bd.com/europe/regulatory/documents.asp#IFU).

Colorless to tan colonies with brownish halos extending into the medium: *Proteus, Morganella, Providencia* strains. Further tests are necessary for complete identification. For details, consult the Instructions for Use of **BBL CHROMagar Orientation** (see: http://www.bd.com/europe/regulatory/documents.asp#IFU).

Rarely, *Pseudomonas aeruginosa* may produce diffusible brown pigment, mimicking *Proteus*. For differentiation, an oxidase test may be performed (see below).

Colorless colonies: Perform an oxidase test: if positive and the typical fruity odor and/or greenish, bluish, or brownish pigmentation (due to the organism's own pigment) is perceived → *Pseudomonas aeruginosa*. It is recommended to use **BD Oxidase Reagent Droppers** (cat. no. 261181) for this test. Perform the oxidase test on filter paper as described in the Instructions for Use of this test, but not on the colonies on the plate. Confirmation by additional tests is recommended.

Pseudomonas aeruginosa will often grow on the opaque side of the plate (= natural resistance); strains with a higher resistance to antibiotics will also grow on the clear side. To determine their exact resistance pattern, all isolates of *P. aeruginosa* from this medium should be tested for susceptibility with approved methods. If oxidase is negative or ambiguous, perform complete biochemical ID. Colorless oxidase negative colorless colonies may include nonfermenters such as *Acinetobacter*, or *Enterobacteriaceae* that do not metabolize any of the included chromogens, such as *Salmonella*. These species may also exhibit resistance to third generation cephalosporins and must not be disregarded!

Mixed cultures on the BBL CHROMagar ESBL (Biplate) plate: they can usually be easily recognized and differentiated from each other by different colony colors. As an example, a

mixed culture of *Klebsiella* and *E. coli* will show blue colonies (*Klebsiella*), and rose to mauve colonies (*E. coli*).
Inspect the plate for the presence of different colony types and colors.
Subcultures on **BBL CHROMagar ESBL (Biplate)** are recommended if more than two different colony types or colors are perceived on the plate.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BBL CHROMagar ESBL (Biplate) is a selective chromogenic screening medium for the direct identification and differentiation of *Enterobacteriaceae* and certain other Gram negative rods resistant to extended spectrum beta-lactams which allows the detection of ESBL producing strains. The medium allows the direct biochemical identification of resistant *E. coli*, and differentiation of other *Enterobacteriaceae* by colony color. Gram positive bacteria and yeast are usually inhibited.
Isolates obtained on this medium must be confirmed to be ESBL producers by additional tests.

Performance Results

In an external performance evaluation, 320 clinical specimens (consisting of 277 rectal swabs, 12 oral/throat swabs, 11 nasal swabs and 20 miscellaneous specimens) were tested on the medium by streaking the swabs from the specimen transport medium directly on the media. Of these 320 specimens, 108 were positive and 212 were negative as determined by the in-house method (automated and manual susceptibility tests including CLSI Confirmatory Test for ESBL producing isolates on Mueller Hinton II Agar.) **On BBL CHROMagar ESBL (Biplate)** a sensitivity of 100% and a specificity of 93% were determined.[6]

Internal Performance Evaluation

Limits of Detection (LOD)
BBL CHROMagar ESBL (Biplate) was evaluated to determine the limit of detection (LOD) of ESBL producing strains. Three test strains (*Klebsiella pneumoniae* ATCC 700603, *E. coli* DSM 22664, and *E. coli* ENF 11013 were evaluated for recovery on **BBL CHROMagar ESBL (Biplate).** Non-selective Columbia Agar with 5% Sheep blood plates were used to determine the organism concentration expressed in colony forming units (CFU) for each dilution. The LOD for **BBL CHROMagar ESBL (Biplate)** ranged from 8-16 CFU at 24 h (average, 13 CFU).

Detection of Resistance
Strains of the following resistance types have been detected on **BBL CHROMagar ESBL (Biplate)**:

Species	Resistance types
Escherichia coli	CAZ-9, TEM-46
	CTX-M, TEM
	CTX-M1
	CTX-M15
	KPC
	OXA-48
	SHV-5; TEM-1b
	TEM
	TEM, SHV
	TEM, CTX-M, SHV
	TEM, SHV, KPC
	TEM, SHV, OXA-1, KPC
	TEM-50
	VIM
Klebsiella oxytoca	VIM
	CTX-M, SHV
Klebsiella pneumoniae subsp. *pneumoniae*	KPC
	NDM-1
	OXA-48
	SHV
	SHV, OXA-10
	SHV-18
	TEM

Species	Resistance types
Enterobacter cloacae	NDM-1
	OXA-2
	SHV
	TEM
	TEM, SHV
	TEM, SHV, OXA-10
	TEM-1, SHV-12
	TEM-1, SHV-5
Salmonella species	SHV
	TEM
Serratia marcescens	TEM, CTX-M, OXA-2
Providencia rettgeri	NDM-1
Non-*Enterobacteriaceae*	
Pseudomonas aeruginosa	IMP
	VIM
	VIM-1
Acinetobacter baumanii	OXA-23
	OXA-58
	VIM-2

	TEM, CTX-M, SHV, IMP-1
	TEM, CTX-M5, SHV
	TEM, SHV
	TEM-1, SHV-5
	TEM-3, SHV
	VIM

This list includes carbapenemase producing strains[2] which grew on the medium in the respective characteristic colony color. Note that tests to confirm carbapenemase production must be applied.

Limitations of the Procedure
Note that both media of this biplate must be inoculated with the same specimen. Do not attempt to inoculate more than one specimen per plate!

Whereas the biochemical identification to the species or group level (based on the chromogenic reactions of the media) is final, the resistance must be confirmed with approved susceptibility testing methods.
Identification of blue, blue-green and colorless isolates to the species level must be performed using biochemical tests.

Certain gram positive bacteria may be resistant to the inhibitors and may grow on the media.

Because the isolation of ESBL producing strains is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage (see **PROCEDURE – Specimen Types**).

A heavy bacterial load and/or some specimens may produce nonspecific coloring of the primary streak area of the media. This could result in the medium exhibiting mauve, purple, green or blue coloration or a slight haze on the medium surface, but lacking distinct colonies. This should be interpreted as negative.

Do not incubate less than 18 hours since this may result in small colonies and / or weak colony coloration; the ideal incubation time is 20 to 22 hours. The incubation should not be longer than 28 hours; in case of mixed cultures, longer incubation may result in coalescing colonies that may be difficult to recognize and purify.

Rarely, ESBL producing strains of *Proteus* spp. will produce weak growth on this medium, especially when present in low CFUs.

It is not recommended to disregard isolates with colorless colonies when screening for ESBL producers on this medium. Perform an oxidase test from these isolates. If this test is negative, perform complete biochemical identification of the isolate.

Although an inhibitor for ampC/ cephalosporinase hyperproducers has been added to the media, a certain percentage of such strains will grow. Therefore, **BBL CHROMagar ESBL (Biplate)** is considered for **screening**, and **not for final identification** of ESBL producers. Specific susceptibility tests or molecular methods are necessary to determine the exact type of resistance expressed by the isolates.

Before using **BBL CHROMagar ESBL (Biplate)** for the first time, we recommend to train the typical colony appearance with defined strains, e.g., the strains mentioned under **USER QUALITY CONTROL**.

REFERENCES

1. Bradford, P.A. 2001. Extended-spectrum ß-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. 14: 933-951.

2. Glasner, C. et al. (2013). Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. Eurosurveillance 18: 1-7.
3. Linscott, A.J. 2007. Specimen collection and transport. *In* L.S. Gracia, and H.D.Isenberg, (eds.), Clinical microbiology procedures handbook, 2nd ed. ASM, Washington DC.
4. Miller, J.M., K. Krisher, and H.T. Holmes. 2007. General principles of specimen collection and handling. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (eds.), Manual of clinical microbiology. 9th ed., ASM, Washington DC.
5. Paterson, D.L., Bonomo, R.A. (2005). Extended-spectrum b-lactamases: a clinical update. Clin. Microbiol. Rev. 18: 657-686.
6. Data on file. Becton Dickinson GmbH.

PACKAGING / AVAILABILITY

BD BBL™ CHROMagar™ ESBL (Biplate)

Cat. No.	**Description**
REF 257606	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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