BD BBL™ CHROMagar™ CPE

INTENDED USE

BD BBL CHROMagar CPE is a selective chromogenic screening medium for the detection of carbapenemase producing *Enterobacteriaceae* (CPE). Appropriate specimens include rectal and perianal 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. Isolates obtained on this medium must be confirmed to be carbapenemase producers by additional tests.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Carbapenem resistence in gram negative bacteria is an increasing problem among nosocomial infections. The resistance can have different reasons but the most common is the worldwide dispersion of bacteria producing carbapenemases that are able to hydrolyze not only carbapenems but also other beta-lactam antibiotics. Genes encoding the carbapenemases are usually located on plasmids which can also be transferred to other species. The diagnostic procedure is complex and it is therefore important to shorten and simplify the detection of these carbapenem resistant bacteria.[1-3]

BBL CHROMagar CPE 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 medium provided in **BBL CHROMagar CPE** allow for the easy detection of mixed cultures of Gram negatives, the identification of *E. coli* (rose to mauve) without further confirmatory tests and for the detection of the *Klebsiella-Enterobacter-Citrobacter-Serratia* (blue to blue-green) and *Proteus-Morganella-Providencia* (colorless to tan and tan to pale blue colonies surrounded by brown halos) and other genera (appearing in their natural colour).
Additionally, **BBL CHROMagar CPE** contains a carbapenem in an appropriate concentration to allow detection of resistance together with other selective agents to inhibit the accompanying flora present in the specimen. Gram negative bacteria such as *Enterobacteriaceae* and non-fermenters, if they are resistant to the antimicrobials included, will produce growth on the medium.

Conventional phenotypic detection of the carbapenemase producing pathogens requires isolation of the strain in pure culture on non-selective media followed by several susceptibility tests to determine the resistance type which is time-consuming and costly.
Using **BBL CHROMagar CPE**, the specimen is streaked onto the medium. After an overnight incubation (18-24 hours), growth of an isolate on the medium is highly conclusive for the presence of *Enterobacteriaceae*. Confirmation by susceptibility tests, molecular methods or phenotypic 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 for detection of CPE.

REAGENTS

BBL CHROMagar CPE

Formula* Per Liter Purified Water

Chromopeptone	16.1 g
Chromogen Mix	1.3
Selective agents	0.23
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 the medium (for details, see **Specimen Types** and **Test Procedure**). Incubate the plates, preferably in an inverted position, at 35 to 37° C aerobically for 18-24 hours.

Strains	**Growth Results**
Klebsiella pneumoniae ATCC BAA-1705 (KPC producer)	Growth moderate to excellent; blue to blue-green colonies
Klebsiella pneumoniae NCTC 13443 (NDM-1 producer)	Growth moderate to excellent; blue to blue-green colonies
Escherichia coli NCTC 13476 (IMP producer)	Growth moderate to excellent; colonies rose to mauve
Klebsiella pneumoniae ATCC 13883	Inhibition complete
Enterococcus faecalis ATCC 29212	Inhibition complete
Staphylococcus aureus ATCC 25923	Inhibition complete
Candida albicans ATCC 60193	Inhibition complete
Uninoculated	Colorless to very light amber, transparent (may contain up to a moderate amount of small particles)

PROCEDURE

Materials Provided

BBL CHROMagar CPE (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 carbapenemase producing strains to aid in the prevention and control of CPE infections in healthcare settings, especially from intensive care units. It is primarily used with rectal and perianal swabs but can be used with

specimens from other body sites suspected to contain carbapenemase producing *Enterobacteriaceae*. Use of transport devices approved for the collection of microbiological clinical specimens is recommended. Follow the transport device manufacturer's recommended procedures.[4,5]
It may also be used for subculturing potential CPE 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
BBL CHROMagar CPE 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 the **BBL CHROMagar CPE** medium and streak for isolation, using a loop. The following procedure for inoculation must be strictly applied to obtain isolated colonies with their typical appearance. Insufficient inoculation or inoculation of the whole medium surface 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.

Inoculation and incubation procedure:

1. Dab the specimen swab on a small area of the **BBL CHROMagar CPE** medium: Do not over-inoculate! Remove the swab from the medium and return the swab to its specimen tube.
2. Using a loop, complete the streaking on the plate. Streak for isolation! First complete the first streak area and streak the second and third area of the medium.
3. Incubate aerobically at 35 to 37° C for 18 to 24 hours, preferably in an inverted position (medium side up). Do not incubate longer and do not incubate in an atmosphere enriched with carbon dioxide.
   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.
4. 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 medium will grow. The plates should show isolated colonies in the areas where the inoculum was diluted appropriately. Appropriate susceptibility tests, molecular methods or phenotypic methods must be performed to confirm the presence of CPE isolates.
Absence of growth on the medium indicates that the specimen does not contain strains with resistance to the antimicrobials included in the medium.
Note that discoloration of the medium without visible colonies (which may occur if the medium has 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 freundii* strains have been found to produce violet to lilac colonies on **BBL CHROMagar CPE**. 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 and tan to pale blue 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.
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 colonies may include nonfermenters such as *Acinetobacter*, or *Enterobacteriaceae* that do not metabolize any of the included chromogens, such as *Salmonella*.

Mixed cultures on the BBL CHROMagar CPE 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 CPE** 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 CPE is a selective chromogenic screening medium for the direct identification and differentiation of carbapenemase producing *Enterobacteriaceae.* 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.[6] Isolates obtained on this medium must be confirmed for carbapenemase production by additional tests.

External Performance Evaluation

In an external performance evalutation, 227 clinical specimens (consisting of 174 rectal and 6 perianal swabs, 10 oral/throat swabs, 9 nasal swabs and 28 miscellaneous specimens) were tested on the medium by streaking the swabs from the specimen transport medium directly on the medium. Of these 227 specimens, 21 were tested positive for CPE and 206 were negative as determined by the in-house method (phenotypic and molecular methods). On **BBL CHROMagar CPE** sensitivity of 100% and a specificity of 94% were determined.[7]

Internal Performance Evaluation

In an internal validation, 274 well-characterized strains from diverse geographically regions were tested. These consisted of 183 CPE strains (including Ambler Class A: 57 KPC, 2 SME; Ambler Class B: 39 NDM, 14 VIM, 11 IMP; Ambler Class D: 53 OXA-48, 1 OXA-162, 2 OXA-163, 4 OXA-181) and 91 non-CPE strains (69 ESBL, 2 Porin deficiencies,12 AmpC, 1 OXY-1, 7 Wild-type). **BBL CHROMagar CPE** demonstrated an overall sensitivity of 94.5% and a specificity of 92.3%. The individual sensitivities for Ambler Class A, B and D carbapenemases were 96.6%, 90.6% and 96.7%, respectively. The medium correctly detected all tested OXA-48 producers (Ambler Class D).[8]

Limit of Detection (LOD)
BBL CHROMagar CPE was evaluated to determine the limit of detection (LOD) of carbapenemase producing strains. Four strains (*K. pneumoniae* NCTC 13438, *K. pneumoniae* NCTC 13443, *E. coli* NCTC 13476 and *E. coli* ENF 18034) were evaluated for recovery on **BBL CHROMagar CPE.** 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 CPE** ranged from 16-31 CFU/ml (average 23,5 CFU/ml) after 24 h of incubation.[8]

Detection of Resistance
Strains of the following resistance types have been detected on **BBL CHROMagar CPE**[6]:

Table 1: Tested strains and resistance types detected on BBL CHROMagar CPE.

Strain	Carbapenemase Class (Ambler Class)	Carbapenemase
Acinetobacter baumanii	Class B	NDM-1, NDM-2
		VIM
		IMP-1
		SIM-1
	Class D	OXA-23, OXA-24, OXA-40, OXA-51, OXA-58, OXA-64, OXA-72, OXA-91, OXA-97, OXA-143
Acinetobacter sp.	Class B	VIM-4
Acinetobacter junii	Class B	IMP-1
Citrobacter freundii	Class A	KPC-2, KPC-3
	Class B	NDM-4
		IMP-4
	Class D	OXA-48, OXA-181
Citrobacter koseri	Class D	OXA-48
Klebsiella pneumoniae	Class A	KPC, KPC-1, KPC-2, KPC-3
	Class B	NDM, NDM-1
		VIM, VIM-1, VIM-4, VIM-19
		IMP-1, IMP-4, IMP-8
	Class D	OXA-48, OXA-162, OXA-163, OXA-181
Klebsiella oxytoca	Class A	KPC-2, KPC-3
	Class B	VIM, VIM-4
Escherichia coli	Class A	KPC, KPC-2, KPC-3, KPC-4, KPC-5
	Class B	NDM, NDM-1, NDM-4
		VIM, VIM-4, VIM-19
		IMP, IMP-1, IMP-8
	Class D	OXA-48
Enterobacter asburiae	Class A	IMI-2
Enterobacter cloacae	Class A	KPC, KPC-2, KPC-3, KPC-4
	Class B	NDM, NDM-1, NDM-4
		VIM-4
		IMP-8
	Class D	OXA-48, OXA-163

Enterobacter sp.	Class B	NDM
Proteus mirabilis	Class B	NDM-1
Providencia rettgeri	Class B	NDM, NDM-1
	Class D	OXA-48, OXA-181
Providencia stuartii	Class B	NDM-1
Pseudomonas aeruginosa	Class A	KPC-2, KPC-5
	Class B	VIM-1, VIM-2, VIM-4, VIM-13
		IMP-7
Salmonella spp.	Class B	IMP-4
Serratia marcescens	Class A	KPC, KPC-2
		SME-1, SME-2
	Class B	IMP-1
	Class D	OXA-48

Limitations of the Procedure
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 medium) is final, the resistance must be confirmed with approved 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 medium. Nonenterobacterial carbapenem-resistant gram negative rods (i.e. *Acinetobacter* spp. and *Pseudomonas* spp.) may grow (appearing in their natural color). It is not recommended to disregard isolates with colorless colonies when screening for carbapenem resistant organisms on this medium. Perform an oxidase test from these isolates. If this test is negative, perform complete biochemical identification of the isolate. For further differentiation, see **PROCEDURE – Results and Interpretation**.

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

Because the isolation of CPE 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 medium. 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 18 to 24 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.

Before using **BBL CHROMagar CPE** 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

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PACKAGING / AVAILABILITY

BD BBL™ CHROMagar™ CPE

Cat. No.	**Description**
REF 257681	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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