

BD BBL™ Selective and Differential Prepared Plated Medium for Gram-Negative Microorganisms MacConkey I Agar

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INTENDED USE

This medium is used in qualitative procedures for the isolation and cultivation of gram-negative enteric microorganisms from a variety of clinical and nonclinical specimens.

Meets USP/EP/JP performance specifications, where applicable.¹⁻³

SUMMARY AND EXPLANATION

BD BBL™ MacConkey I Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.⁴ Bile salts and crystal violet are employed as the selective agents. Neutral red is incorporated to differentiate lactose fermenters from lactose nonfermenters.

PRINCIPLES OF THE PROCEDURE

Selective and differential media have long been employed in the isolation, enumeration and identification of enteric microorganisms, especially enteric pathogens. These selective media contain bile salts, defined chemicals, dyes or other agents, which inhibit the growth of undesired organisms.

Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

REAGENTS

Formula:

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin.....	17.0	g
Peptones (meat and casein)	3.0	g
Lactose	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Neutral Red	0.03	g
Crystal Violet	0.001	g
Agar	13.5	g

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

Warnings and Precautions:

For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{1-3,5-8}

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁹⁻¹² and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

PROCEDURE

Material Provided: BD BBL MacConkey I Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms, and laboratory equipment as required.

Test Procedure: Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture.

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. A nonselective medium should also be streaked 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.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2 °C (do not use CO₂-enriched atmosphere with **BD BBL MacConkey Agar**) or other appropriate temperature for 18–24 h.

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 give known, desired reactions. The following test strains are recommended:

For clinical and other non-USP/EP/JP applications:

Test Strain	Expected Results
<i>Escherichia coli</i> ATCC® 25922	Growth, pink colonies
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium ATCC 14028	Growth, colorless colonies
<i>Enterococcus faecalis</i> ATCC 29212	Inhibition (partial)

For USP/EP/JP applications*:

Test Strain	Inoculum	Incubation	Expected Results
<i>Escherichia coli</i> ATCC 8739	<100 colony-forming units (CFU)	30–35 °C for 18–72 h	Growth comparable to previously accepted lot of medium

*For USP/EP/JP applications, inoculum level and incubation conditions are prescribed.

RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the selective media used.

Typical colony morphologies and reactions on **BD BBL MacConkey I Agar** are:

E. coli.....Pink to rose-red (may be surrounded by a zone of precipitated bile)

Enterobacter.....Mucoid pink
and *Klebsiella*

Salmonella.....Colorless

Shigella.....Colorless

Pseudomonas.....Irregular, colorless to pink; a green to yellow-green pigment may be produced in areas of heavy growth

Gram-positiveNo growth to slight growth

LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended as a primary medium for inoculation; some diagnostic tests may be performed with the primary plates. For identification, the organisms must be in pure culture. Biochemical and serological tests may be performed for complete identification. Consult appropriate texts for detailed information and recommended procedures.^{6,8,13,14}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on **BD BBL** MacConkey Agar when incubated in a CO₂ atmosphere (G. Mazura-Reetz, T.R. Neblett, and J.M. Galperin. Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C179, p. 139).

PERFORMANCE CHARACTERISTICS

Prior to release, all lots of **BD BBL** MacConkey I Agar are tested for performance characteristics. Representative samples of the lot are streak-inoculated with the following cultures: *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella* Typhimurium (ATCC 14028), *Shigella dysenteriae* (ATCC 9361), and *Enterococcus faecalis* (ATCC 29212). The inoculum for all organisms is diluted to yield 10³–10⁴ CFUs/plate with the exception of *E. faecalis* which is diluted to yield 10⁴–10⁵ CFUs/plate. After inoculation, the plates are incubated at 35 ± 2 °C in an aerobic atmosphere. After 18–24 h of incubation, *E. coli* exhibits moderate to heavy growth, colonies are pink to rose-red and may be surrounded by precipitated bile; *P. mirabilis* exhibits fair to heavy growth of colorless colonies and may exhibit swarming; *P. aeruginosa* yields fair to heavy growth and may exhibit green to yellow-green pigmentation while individual colonies show pink to green pigmentation; *S. Typhimurium* yields fair to heavy growth of colorless colonies; *S. dysenteriae* shows fair to heavy growth of colorless colonies to pink colonies; and *E. faecalis* is completely to partially inhibited (fair growth) and the colonies may be pink in color.

AVAILABILITY

Cat. No. Description

215197 **BD BBL™** MacConkey I Agar, Pkg. of 20 plates

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