# BD BBL™ MacConkey II Agar

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## QUALITY CONTROL PROCEDURES

### I. INTRODUCTION

MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

### II. PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation. Use cultures diluted to yield $10^3$-$10^5$ CFU/plate.
   b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA5%SB) plates as nonselective controls for all organisms.
2. Examine plates after 18-24 h for growth, pigmentation and selectivity.
3. Expected Results

| CLSI Organisms | ATCC™ | Recovery | Colony Color |
|---|---|---|---|
| **Escherichia coli* | 25922 | Growth | Pink |
| **Proteus mirabilis* | 12453 | Growth<br>Inhibition of swarming (partial) | Colorless |
| **Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium | 14028 | Growth | Colorless |
| **Enterococcus faecalis* | 29212 | Inhibition (partial) | May be pink |
| **Additional Organisms** | | | |
| *Pseudomonas aeruginosa* | 10145 | Growth | Pink to green |
| *Shigella dysenteriae* | 9361 | Growth | Colorless to pink |

*Recommended organism strain for User Quality Control.

### III. ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1°C for 60 h and examine for microbial contamination.

## PRODUCT INFORMATION

### IV. INTENDED USE

MacConkey II Agar is a selective and differential medium for the detection of coliform organisms and enteric pathogens.

### V. SUMMARY AND EXPLANATION

At the present time, many culture media are available to the laboratorian for the isolation, cultivation and identification of enteric bacteria. One of the earliest of these was developed by MacConkey and first described as a brief published note.[1] The landmark paper on MacConkey Agar was published in 1905 and contained detailed descriptions of the medium and the bacterial growth patterns obtained.2 This formulation was devised in the knowledge that bile salts are precipitated by acids and certain enteric microorganisms ferment lactose whereas others do not possess this ability.

Since the publication of the early papers, the MacConkey Agar formula has been modified many times. A

compilation of culture media published in 1930 lists ten modifications which were published up to that time.[3] More recent modifications include use of additives (e.g., kanamycin) and the deletion of certain ingredients (e.g., crystal violet, and neutral red[4]).

MacConkey Agar is recommended for use with clinical specimens likely to contain mixed microbial flora, such as urine, respiratory and wound, because it allows a preliminary grouping of enteric and other gram-negative bacteria.[5,6] MacConkey Agar is also used in the BAM (*Bacteriological Analytical Manual*) of the Food and Drug Administration (FDA) procedure for isolating *E. coli* from foods.[7]

The **BBL** MacConkey II Agar formulation was made available in 1983. It was specially designed to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for the promotion of superior growth of enteric pathogens.

## VI. PRINCIPLES OF THE PROCEDURE

MacConkey II Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibits gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci.

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.

## VII. REAGENTS

**MacConkey II Agar**

Approximate Formula* Per Liter Purified Water

| | |
|---|---|
| Pancreatic Digest of Gelatin | 17.0 g |
| Pancreatic Digest of Casein | 1.5 g |
| Peptic Digest of Animal Tissue | 1.5 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 in Taiwan

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.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[8-11] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**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.

## VIII. SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[12,13] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX. PROCEDURE

**Material Provided:** MacConkey II 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 $CO_2$-enriched atmosphere with MacConkey II Agar) or other appropriate temperature for 18 - 24 h.

**User Quality Control:** See "Quality Control Procedures."

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. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

## X. 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 medium.

Typical colonial morphology on MacConkey II Agar is as follows:

| | |
|---|---|
| *E. coli* ..... | Pink to rose-red (may be surrounded by a zone of precipitated bile) |
| *Enterobacter/Klebsiella*..... | Mucoid, pink |
| *Proteus* ..... | Colorless, swarming in areas of isolated colonies is inhibited |
| *Salmonella* ..... | Colorless |
| *Shigella* ..... | Colorless |
| *Pseudomonas*..... | Irregular, colorless to pink |
| Gram-positive bacteria ..... | No growth to slight growth |

## XI. LIMITATIONS OF THE PROCEDURE

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO2-enriched atmosphere.[13]

Not all strains of *E. coli* ferment lactose.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[5,12,14-17]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

## XII. AVAILABILITY

| Cat. No. | Description |
|---|---|
| 251270 | BD **BBL™** MacConkey II Agar, Ctn. of 100 plates |

## XIII. REFERENCES


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## XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

Nippon Becton Dickinson Company, Ltd.
1 Aza Gotanda, Tsuchifune, Fukushima City, Fukushima, Japan
e-mail : BD-eDial@bd.com
WEB : http://www.bd.com/jp/