



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Moeller KCN Broth Base, when supplemented with potassium cyanide, is useful in differentiating organisms based on their ability to grow in the presence of cyanide.

**CAUTION: Do not pipette KCN solution by mouth. Use a syringe or bulb pipette. Cyanide is extremely poisonous and can cause death instantly. Do not inhale fumes. Work under a chemical hood.**

II PERFORMANCE TEST PROCEDURE

(Testing of the base without supplementation with KCN)

1. Inoculate representative samples with the cultures listed below.
  - a. Inoculate the tubes with  $10^3$ – $10^4$  CFU using dilutions of 5-h **Trypticase™** Soy Broth cultures.
  - b. Incubate tubes with loosened caps at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere.
2. Examine tubes after 18–24 and 42–48 h for growth.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Citrobacter freundii</i>	8454	Growth
<i>Enterobacter aerogenes</i>	13048	Growth
<i>Escherichia coli</i>	25922	Growth
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth

\*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes 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  $7.6 \pm 0.2$ .
4. Incubate uninoculated representative tubes aerobically at  $20$ – $25^\circ\text{C}$  and  $35$ – $37^\circ\text{C}$  and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Moeller KCN Broth Base, when supplemented with a solution of potassium cyanide, is used in the differentiation of enteric bacilli on the basis of their ability to grow promptly in the presence of cyanide.

V SUMMARY AND EXPLANATION

In 1954, Moeller reported on the use of a medium containing cyanide as an aid in the differentiation of members of the *Enterobacteriaceae*.<sup>1</sup> Edwards and Ewing<sup>2</sup> modified Moeller Cyanide Broth and it is this modified formulation that is supplied by BD Diagnostics as Moeller KCN Broth Base prepared in a tube.

This medium, when supplemented with potassium cyanide, is used in the differentiation of members of the genus *Salmonella* (including the Arizona group) from *Citrobacter freundii*.<sup>3</sup> It is particularly useful in differentiating cultures of *C. freundii* that either fail to ferment lactose or ferment it slowly (i.e., strains formerly classified as Bethesda-Ballerup bacteria).<sup>3</sup> Except for group IV and V strains, which are insignificant in terms of human disease, members of the genus *Salmonella* do not grow in KCN medium, whereas species of *Citrobacter* (with the exception of *C. diversus*) do grow in this medium.<sup>3,4</sup>

VI PRINCIPLES OF THE PROCEDURE

The addition of 0.15 mL of a 0.5% solution of potassium cyanide to each of the prepared tubes of the nutritive base enables differentiation of members of various genera within the *Enterobacteriaceae* family.

VII REAGENTS

Moeller KCN Broth Base

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	1.5	g
Peptic Digest of Animal Tissue .....	1.5	g
Sodium Chloride .....	5.0	g
Monopotassium Phosphate .....	0.225	g
Disodium Phosphate .....	5.64	g

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

**Warnings and Precautions:** For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store tubes in the dark at  $2$ – $25^\circ\text{C}$ . Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

## VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

## IX PROCEDURE

**Material Provided:** Moeller KCN Broth Base

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

**Test Procedure:** Observe aseptic techniques.

Prior to use, add 0.15 mL of a 0.5% solution (0.5 g in 100 mL of *COLD* sterile distilled water) of potassium cyanide to each tube containing 10 mL of base and close tightly.

**PRECAUTION: Do not mouth pipette. Extreme care should be taken at all times in handling and disposing of potassium cyanide. Work should be performed within a chemical hood.**

Inoculate Moeller KCN Broth with a loopful of an 18- to 24-h culture of the test organism. Incubate at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere and examine daily for 2 days.

**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.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of the broth medium.

## X RESULTS

Except for a few infrequently-isolated species, members of the genera *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus* and *Providencia* are KCN positive; i.e., they grow in the presence of KCN. With certain exceptions, other *Enterobacteriaceae* are negative (inhibited) in the KCN test.<sup>4,5</sup>

## XI LIMITATIONS OF THE PROCEDURE

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.<sup>4-9</sup>

## XII AVAILABILITY

Cat. No.	Description
221665	BBL™ Moeller KCN Broth Base, 10 mL, Pkg. of 10 size K tubes

## XIII REFERENCES

1. Moeller, V. 1954. Diagnostic use of the Braun KCN test within the *Enterobacteriaceae*. *Acta Pathol. Microbiol. Scand.* 34:115-126.
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5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
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7. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
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9. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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