

ESCHERICHIA COLI
(CULTURE METHOD)

PRINCIPLE

Escherichia coli is isolated from samples by using an enrichment procedure with a selective medium. It is confirmed through the use of a selective and differential medium in conjunction with a biochemical test.

SCOPE

The method is applicable to starches, sugars, syrups and most co-products of the corn wet milling industry (Note 1).

SPECIAL APPARATUS

1. Long wavelength UV lamp
2. Test tubes containing gas collector tubes (Durham tubes)

MEDIA AND REAGENTS

1. Lactose Broth (LB). Prepare medium according to manufacturer's directions. Dispense 100 mL into 250 mL Erlenmeyer flask and sterilize by autoclaving at 121°C for 15 minutes.
2. MacConkey Agar (MA). Prepare medium according to manufacturer's directions. Sterilize by autoclaving at 121°C for 15 minutes. Cool agar to 45-50°C, dispense 15 mL of sterile medium into Petri dishes and let solidify.
3. Lauryl Tryptose Broth with MUG (LTB-MUG) (Note 2). Prepare medium according to manufacturer's directions. Dispense 10 mL into culture tubes (20 × 150 mm) containing the inverted gas collector tubes. Sterilize by autoclaving at 121°C for 15 minutes.

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PROCEDURE

Aseptically weigh 10 g of sample into LB, mix well and incubate for 46-50 hours at 35-37°C. Using a 3 mm inoculating loop, streak from LB onto a MA plate and at the same time, transfer a loopful of suspension into tube of LTB-MUG (Note 3). Incubate the inverted MA plate and LTB-MUG tube for 24 to 48 hours at 35-37°C. Examine the MA plate for brick-red colonies (lactose positive fermenter) and the MUG tube for fluorescence under UV light (Note 4). Bluish fluorescence is positive for MUG hydrolysis.

CALCULATION

Report positive *E. coli* per 10 g sample if the presence of brick-red colonies is supported by a positive MUG test (Note 4).

NOTES AND PRECAUTIONS

1. Since the MUG test is not an official method for *E. coli*, it should not be used for analysis of regulatory samples. The conventional method based on IMViC test (Indole, Methyl Red, Voges-Proskauer, Citrate) is recommended for such samples.
2. MUG is an abbreviation for 4-methylumbilliferyl-beta-D-glucuronic acid.
3. The test should be run with a positive control. A 10 g sample is required for USP testing and 1 g for food standards.
4. The MUG test is based on the detection of beta glucuronidase activity, which is present in most *E. coli* (97%). It is also present in some *Salmonella*, *Shigella* and *Yersinia*. However, these are non-lactose fermenting microorganisms, which can be easily differentiated from *E. coli*. Tubes of sterile LTB-MUG or equivalent media are also available commercially. These ready-to-use tubes are equally reliable for the positive identification of *E. coli*.

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.