ColiComplete®

AOAC Official Method 992.30

General Description

ColiComplete® contains 5-bromo-4-chloro-3-indolyl-ß-Dgalactopyranoside (X-Gal) and 4-methyl umbelliferyl-ß-D-glucuronide (MUG). Discs are added to LST inoculated with selected dilutions of samples. Samples are incubated at 35–37 °C and examined after 24 and 48 ±2 h for confirmed total coliforms and after 30 ±2 h for confirmed *E. coli* results. ß-Galactosidase, from coliforms present in samples, cleaves X-Gal into 5-bromo-4-chloro-indoxyl intermediate which undergoes oxidation to yield water-insoluble blue dimer, visually detectable on disc or in surrounding medium as confirmed positive result for total coliform activity. ß-Glucuronidase, from *E. coli* present in samples, cleaves MUG into glucuronide and methyl umbelliferone which fluoresces under long wave UV light (366 nm) as confirmed positive result for *E. coli* presence.

NOTE: As *E. coli* O157:H7 does not produce ß-glucuronidase, ColiComplete® is not suitable for the detection of *E. coli* O157:H7.

A. Sample Preparation

Prepare appropriate serial dilutions as indicated in FDA Bacteriological Analytical Manual (BAM), or AOAC Official Methods of Analysis according to sample type.

B. Inoculation

Inoculate LST tubes with appropriate sample dilution series selected to determine MPN levels or presence/absence of total coliforms and E. coli in sample. Aseptically add a single ColiComplete® disc to each tube. Incubate at 35–37 °C.

C. Reading ColiComplete®

a. For total coliforms — After at least 24 h incubation, examine each tube for visually detectable blue color on disc or in surrounding medium. Presence of blue color indicates confirmed positive result for total coliforms.

NOTE: A wide range of blue color intensity may be expected, depending on sample composition and microflora. All blue reactions are positive regardless of intensity of color.

Reincubate at 35–37 °C. After additional 24 ±2 h re-examine. Continued absence of blue indicates negative result; presence of blue indicates confirmed positive result for total coliforms. Read and record the MPN code or presence/absence of total coliforms in the sample.

b. For *E.coli* — After 30 ±2 h from start of initial incubation, examine tubes under long-wave UV light (366 nm). Fluorescent tubes indicate confirmed positive result for E. coli. Read and record the MPN code or presence/absence of E. coli in the sample.

D. CONTROLS

Positive and negative controls should be used to facilitate interpretation of MUG fluorescent reaction. Use one known positive *E. coli* tube and two negative controls - one non -*E. coli*/coliform tube (e.g., Klebsiella spp.) and one uninoculated media tube.

NOTE: Use borosilicate glass tubes, flint glass gives fluorescence that may be misinterpreted for a positive result.

E. Method Modification for Certain Juices

Applicable to juice products/processors which rely on treatments that do not come into direct contact with all parts of the juice, as contained in 21 CFR Part 120: Rules and Regulations. Hazard Analysis and Critical Control Point (HAACP); Procedures for the Safe and Sanitary Processing and Importing of Juice; Final Rule. Vol 66 No. 13. 6137-6202. Use the modified method “Analysis for Escherichia coli in Citrus Juices - Modifi cation of AOAC Official Method 992.30” as stated in Section 120.25 (a).

F. Storage

Store unused discs at 2–8 °C (36–46 °F) in a sealed container, with desiccant.

G. Disposal

After use, all tubes must be steam-sterilized at 121 °C for at least 30 min before discarding. For in-vitro diagnostic use only.

Manufacturing Entity

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