

# ColiComplete®

AOAC Official Method 992.30

## General Description

ColiComplete® contains 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) and 4-methyl umbelliferyl- $\beta$ -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  $\pm$  2 h for confirmed total coliforms and after 30  $\pm$  2 h for confirmed *E. coli* results.  $\beta$ -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.  $\beta$ -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  $\beta$ -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  $\pm$  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  $\pm$  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.

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## 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 - Modification 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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