IDEXX Summary 5I-v2

Topic: AOAC inclusion of Colilert® as an approved method (991.15) for the detection and enumeration of total coliforms and E coli in water

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Highlights:

- Colilert is listed in section 17.3 as AOAC Method 991.15
- Colilert is used for the detection of total coliforms and *E coli* in water by either the Presence/Absence or MPN enumeration method
- Colilert is described as "Defined Substrate Technology (DST) reagent system simultaneously enumerates total coliforms and E coli directly and separately from a water test sample

AOAC Official Method 991.15
Total Coliforms and *Escherichia coli* in Water
Defined Substrate Technology (Colilert) Method
First Action 1991
Final Action 1994

Results of the interlaboratory study supporting the acceptance of the method:

6.4 bacteria/100 mL (geom. mean 21.88; log geom. mean 1.34):
$s_r = 0.27$; $s_R = 0.35$; $RSD_r = 20.15\%$; $RSD_R = 26.12\%$

39 bacteria/100 mL (geom. mean 93.33; log geom. mean 1.97):
$s_r = 0.32$; $s_R = 0.37$; $RSD_r = 16.24\%$; $RSD_R = 18.78\%$

81 bacteria/100 mL (geom. mean 154.88; log geom. mean 2.19):
$s_r = 0.20$; $s_R = 0.39$; $RSD_r = 9.13\%$; $RSD_R = 17.81\%$

A. Principle

Defined substrate technology (DST) reagent system simultaneously enumerates total coliforms and *E. coli* directly and separately from a water test sample. Reagent contains *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). After inoculation of DST test, a clear solution results. Only total coliforms can hydrolyze ONPG to produce yellow chromogen. Same test tube or vessel contains MUG, which is hydrolyzed and fluoresces when *E. coli* grow. β-Glucuronidase has been found specific to the genus *Escherichia* (*Escherichia* and *Shigella*) and *Salmonella*. Practically, from water samples, only *E. coli* yields a positive result. Metabolism of ONPG by β-D-galactosidase system of enteric bacteria is specific for total coliform group. Composition of inorganic salts in DST reagent does not support growth of nonenteric bacteria. Assay may be performed in most probable number (MPN) format or as presence-absence (P-A) test.

B. Apparatus

(**a**) *Tubes.*—Glass, 12 mL. Sterile, free of microbial inhibitors (e.g., residual detergent), and nonfluorescent at 366 nm.

(**b**) *Vessels.*—Glass, 120 mL. Sterile, free of microbial inhibitors (e.g., residual detergent), and nonfluorescent at 366 nm.

(**c**) *Longwave ultraviolet light source.*—366 nm, 4 watt, hand-held lamp (UVP, Inc., San Gabriel, CA, USA) or equivalent.

C. Reagent

For each 1000 mL test sample, completely mix the following: ammonium sulfate, 5 g; manganese sulfate, 50 mg; zinc sulfate, 50 mg; magnesium sulfate, 100 mg; sodium chloride, 10 g; calcium chloride, 50 mg; potassium dihydrogen phosphate, 900 mg; disodium hydrogen phosphate, 6.2 g; sodium sulfate, 40 mg; amphotericin B, 1 mg; ONPG, 500 mg; MUG, 75 mg; and solanium, 500 mg.

D. Enumeration

For MPN format, use sufficient reagent mixture (*see* **C**) in each tube to accept 10 mL test portion; for P-A format, use 10 times that amount in each vessel. If laboratory-prepared reagent is used, add powder to labeled tube, **B(a)**, or vessel, **B(b)**, containing test portion. Or, add well-mixed water test portion to labeled tube or vessel containing predispensed reagent. Combine test portion and reagent aseptically, cap container tightly, and mix vigorously to dissolve reagent. Resulting solution is colorless. Incubate samples for 24 h at 35 ± 1.0°C. Yellow color in MPN tube or P-A vessel after incubation denotes presence of total coliforms. Expose positive total coliform tubes or vessel to hand-held 366 nm lamp, **B(c)**. Fluorescence denotes presence of *E. coli.*

Use standard MPN tables, Table **966.24A** (*see* 17.2.02) or Table **978.23** (*see* 17.3.05), to determine MPN values. Report results as total coliform MPN/100 mL test sample or *E. coli* MPN/100 mL test sample.

E. Quality Control

Perform quality control as follows:

(*1*) Reconstitute reagent in each of 3 tubes or vessels with appropriate volume of sterile, distilled water and mix thoroughly to aid dissolution.

(*2*) Label tubes "*Escherichia coli*," "*Klebsiella pneumoniae*," and "*Pseudomonas aeruginosa.*"

(*3*) Touch sterile inoculating loop or needle to an 18–24 h pure culture slant of each of the 3 bacteria (alternatively, a "Bactrol," or equivalent, disc of 3 respective bacteria may be used directly).

(*4*) Transfer each bacterial inoculum to appropriately labeled tube or vessel.

(*5*) Incubate inoculated tube or vessel for 24 h at 35 ± 1.0°C. Results should be *E. coli*, yellow and fluorescent; *K. pneumoniae*, yellow only; and *P. aeruginosa*, no color and no fluorescence.

Reference: *JAOAC* **74**, 526(1991).