



Standard Operating Procedure

R-CARD® ECC (Duogen™ Technology)

Rapid Test Method for *E. coli* and Coliform

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1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD™ ECC (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of *Escherichia coli* (*E. coli*) and coliform within 15 to 20 hrs. Because these bacteria are natural inhabitants of the intestinal tract of warm-blooded animals, their presence in water and food samples are an indication of fecal pollution and the possible presence of other enteric pathogens. This test for *E. coli* and coliform can be applied to water, food or others. **(NOTE: If the sample contains *Aeromonas spp.*, they may give a similar appearance to the true coliform)**
- 1.2 The detection limit is one colony forming unit (CFU) per sample.

2. Summary of Method

- 2.1. A liquid sample is pipetted on the center of the card, and slowly covered by the top film. The liquid sample will spread laterally automatically within 1 min. The card is then incubated at 35±0.5°C for 15 -20 hr. In ambient light, blue to dark purple and dark pink/red colonies are counted as *E.coli* and coliforms, respectively.

3. Method Definition

- 3.1. In this method, *E. coli* are those bacteria which produce blue to dark purple colonies and other coliforms will produce light to dark pink/red colonies on R-CARD™ ECC between 15-20 hr incubation.
- 3.2 R-CARD™ ECC is ready-to-use for detecting *E. coli* and other coliforms in liquid samples.

4. Interferences

- 4.1. If the liquid sample is too turbid, it may become difficult to observe light colored colonies (coliforms).

5. Safety

- 5.1. Analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials and while operating sterilization equipment.
- 5.2. Mouth-pipetting is prohibited.

6. Equipment and Supplies

- 6.1. Sterile pipettes (1 to 25 mL)
- 6.2. Forceps: smooth, flat, sterilizable metal forceps.
- 6.3. Microscope: A 10 to 15 X magnification binocular wide-field dissecting microscope.
- 6.4. Light box
- 6.5. Bunsen burner or alcohol lamp for sterilizing forceps if necessary.

7. Reagents and Standards

- 7.1. Sterile deionized or distilled water
- 7.2 R-CARD™ ECC

8. Quality Assurance/Quality Control

- 8.1. Quality control
 - 8.1.1. Each lot of R-CARD™ ECC medium should be evaluated by the laboratory by preparing three plates of the medium (one to serve as an uninoculated control, one to serve as a negative growth control, and one to serve as positive control).
 - 8.1.2. 8.1.2 *E. coli* ATCC #11775 or 25922 is used as the positive control. *Enterobacter aerogenes* ATCC 13048 is used as the coliform positive control, and *Pseudomonas aeruginosa* ATCC 10145 or 27853 is used as the negative growth control microorganism.

9. Procedure

- 9.1. Prepare samples as usual and make a serial dilution if necessary.
- 9.2. Wear glove and open the top portion (film) or use sterile forceps (see photos 1-2)
- 9.3. Select dilutions of the sample to produce 20-150 coliform/*E. coli* colonies on the cards.
- 9.4. Pipette 1 mL of the sample on the center of the card (photo 3).
- 9.5. Cover the film, and wait 1 min to allow liquid to spread automatically. There is no need to use a spreader. (photo 4).
- 9.6. Incubate at $35\pm 0.5^{\circ}\text{C}$ for 15-20 hrs (no more 24 hrs). Incubation may be at $44.5\pm 0.2^{\circ}\text{C}$ for detecting *E. coli* only.

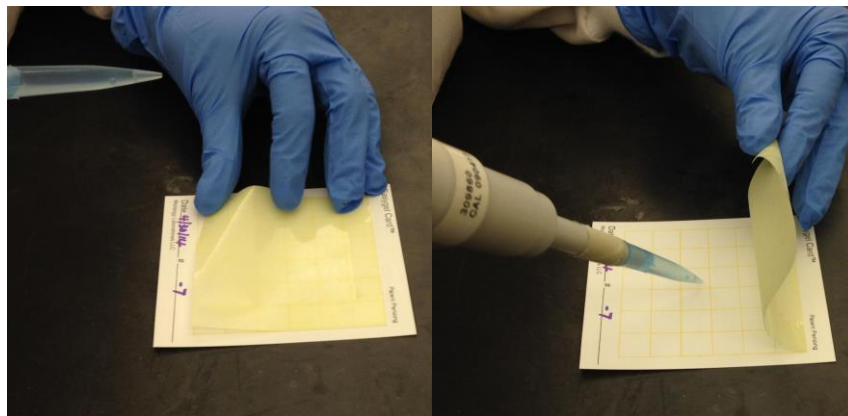


Photo 1. Open the film

Photo 2. Lift the film

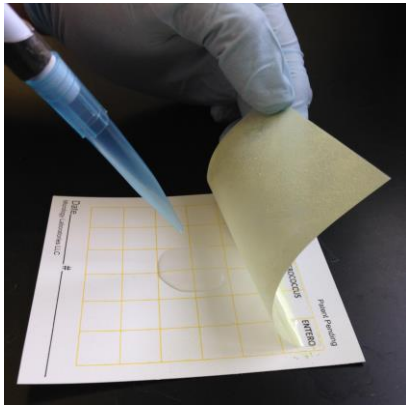


Photo 3. Pipette 1 mL sample

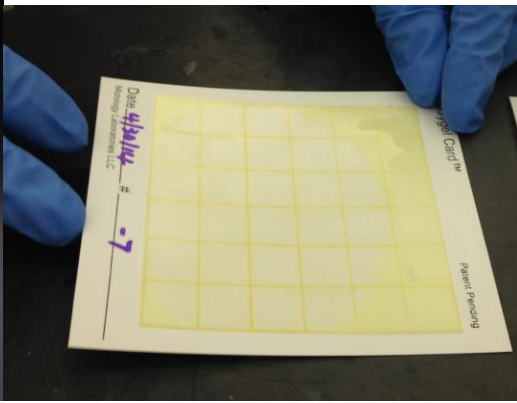
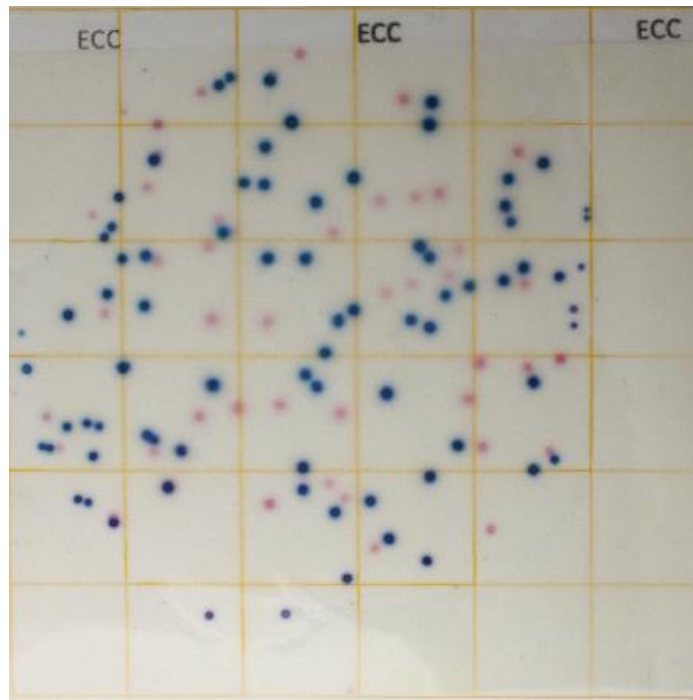


Photo 4. Cover the film

10. Data Analysis and Calculations

- 10.1. Count the number of colonies detected by dark blue to purple or light to dark pink/red colonies present on the card between 15-20 hr incubation and record as the number of *E. coli*/coliform/volume of sample for that test.

Dark blue to purple colonies for *E. coli* and light to dark pink/red colonies for other coliforms



11. Pollution Prevention and Waste Management

- 11.1. All biohazardous waste should be sterilized at 121°C for 30 min prior to disposal. Laboratory personnel should use pollution control techniques to minimize waste generation wherever possible.