



Standard Operating Procedure

R-CARD® E. Coli 3mL Capacity

Rapid Test Method for *Escherichia coli* (*E. coli*)

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1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD® E. Coli (Roth Bioscience, LLC) for detection and enumeration of *Escherichia coli* (*E. coli*) within 15 to 24 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* can be applied to water, food or others
- 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 $35\pm 0.5^{\circ}\text{C}$ for 15 -24 hr. Under ambient light, teal/green colonies are counted as *E.coli*.

3. Method Definition

- 3.1. In this method, *E. coli* are those bacteria which produce teal/green colonies on the R-CARD® E. Coli between 15-24 hr incubation.
- 3.2. R-CARD® E. Coli is ready-to-use for detecting *E. coli* in liquid samples.

4. Interferences

- 4.1. If the liquid sample is too turbid, it may become difficult to observe teal/green colonies.

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® E. Coli

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of the R-CARD® E. Coli 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. *E. coli* ATCC #11775 or 25922 is used as the positive control. *Klebsiella pneumoniae* ATCC 31488 is used as the negative control, and also *Pseudomonas aeruginosa* ATCC 10145 or 27853 may be used as a 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 *E. coli* colonies on the cards.

9.4. Pipette 3 mL of the sample on the center of the card (photo 3).

9.5. Cover with the film, and wait 1 min to allow liquid to spread automatically. There is no need to use a spreader. (photo 4). (Some samples do not automatically spread in as large an area as may be wanted, but it is a simple matter to encourage spreading by gently applying pressure on the top after it is lowered on the inoculum.)

9.6. Incubate at $35\pm 0.5^{\circ}\text{C}$ for 15-24 hrs (no more 24 hrs). Incubation may be as high as $44.5\pm 0.2^{\circ}\text{C}$ for detecting only *E. coli*, as it is more temperature tolerant than most other *Coliforms*.

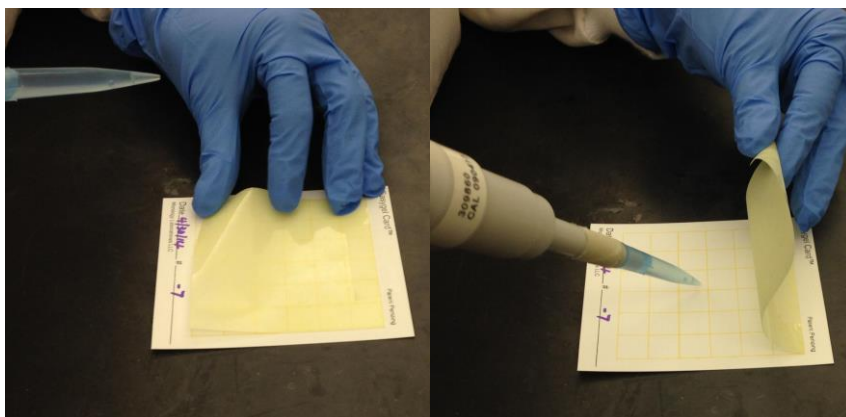


Photo 1. Open the film

Photo 2. Lift the film

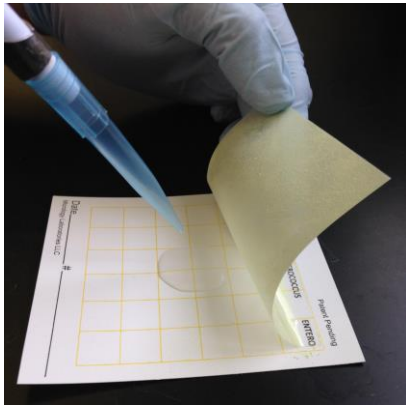


Photo 3. Pipette 1 mL sample

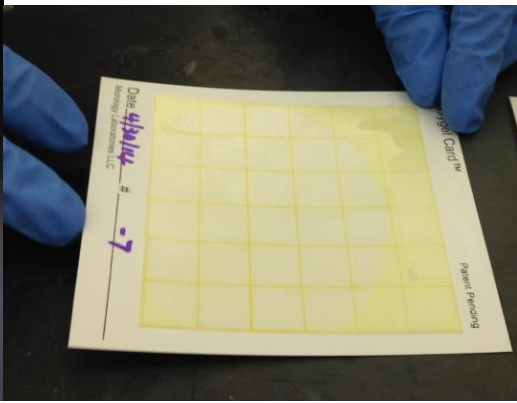
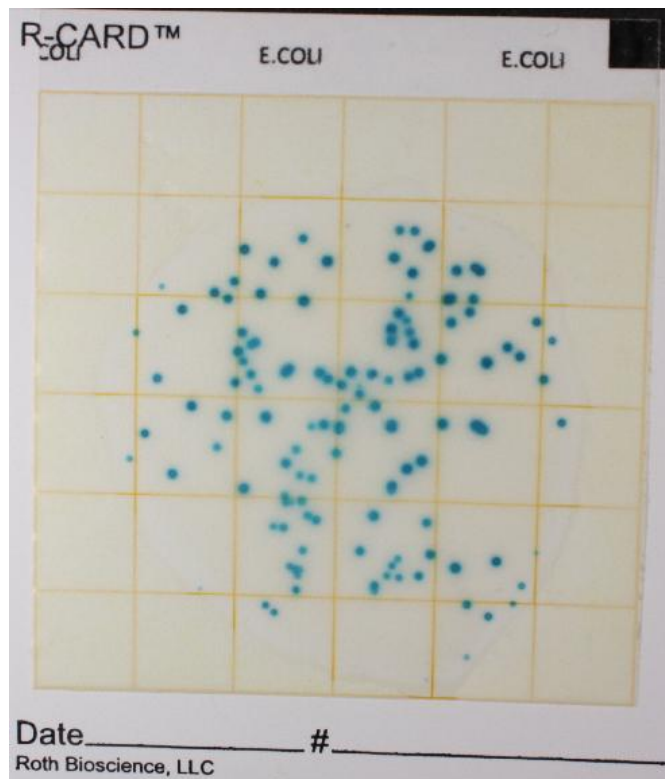


Photo 4. Cover with the top film

10. Data Analysis and Calculations

- 10.1. Count the number of colonies detected by green/teal colonies present on the card between 15-24 hr incubation and record as the number of *E. coli*/volume of sample for that test.



Green/turquoise colonies are counted as *E. coli*.

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.