



Standard Operating Procedure

R-CARD™ Coliform

Rapid Test Method for Coliform Bacteria

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1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD™ (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of coliforms (*Escherichia coli* is a 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 Coliform 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 $35\pm0.5^{\circ}\text{C}$ for 15 -20 hr. Under an ambient light, Green/turquoise colonies are counted as coliform.

3. Method Definition

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

4. Interferences

- 4.1. If the liquid sample is too turbid, it may become difficult to observe green/teal coliform 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. The R-CARD™ Coliform

8. Quality Assurance/Quality Control

8.1. Quality control

- 8.1.1. Each lot of the R-CARD™ Coliform 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. Coliform 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 coliform colonies on the cards.
- 9.4. Pipette 1 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 15-20 hrs (no more than 24 hrs). Incubation may be as high as $44.5 \pm 0.2^\circ\text{C}$ for isolating only *E. coli*, which is tolerant of the higher temperature.

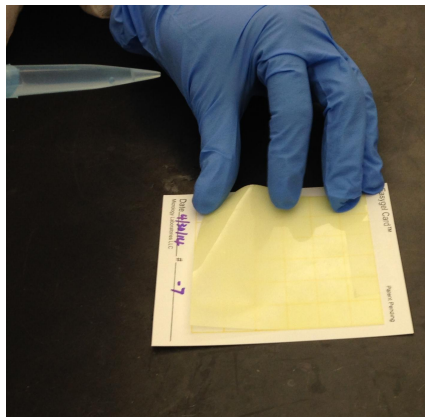


Photo 1. Open the film

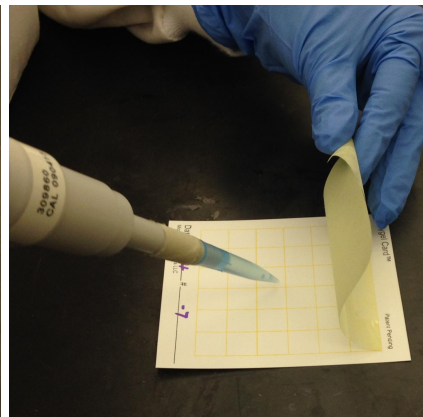


Photo 2. Lift the film

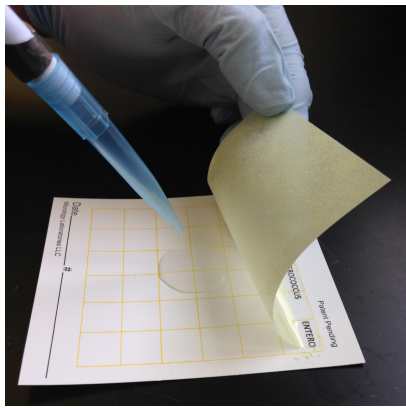


Photo 3. Pipette 1 mL sample

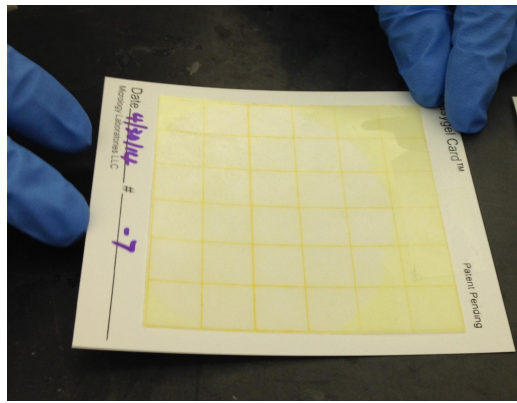
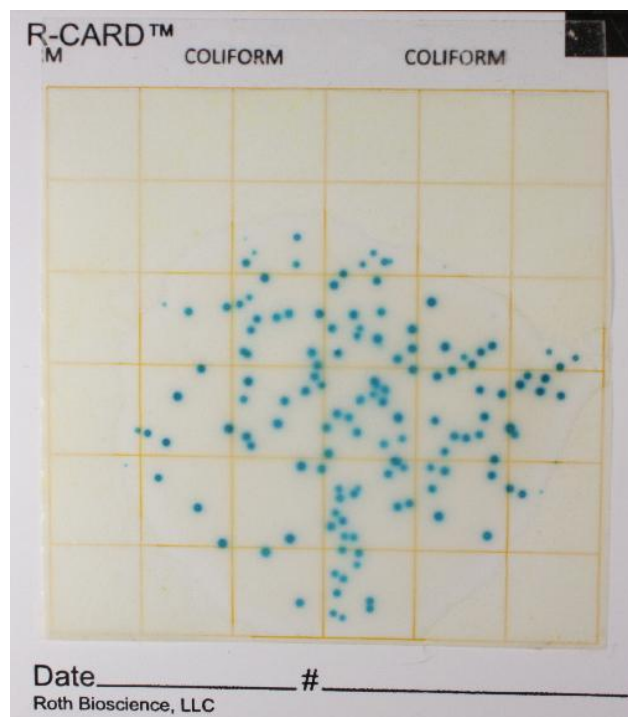


Photo 4. Cover the film

10. Data Analysis and Calculations

- 10.1. Count the number of colonies detected by green/teal colonies present on the card between 15-20 hr incubation and record as the number of Coliform/volume of sample for that test.



Green/teal colonies are counted as Coliform

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