



Standard Operating Procedure

R-CARD® Enterococcus

Rapid Detection of *Enterococcus faecalis*

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1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD® Enterococcus (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of *Enterococcus faecalis* bacterium within 15 to 36 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 *Enterococcus faecalis* can be applied to water, food or others, but is favored by some investigators for the identification of fecal contamination in marine waters.
- 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 -36 hr.in ambient light, green/teal colonies are counted as *Enterococcus faecalis*, and counted as a fecal indication of contamination.

3. Method Definition

- 3.1. In this method, *Enterococcus faecalis* are those bacteria which produce green/teal colonies on R-CARD® Enterococcus between 15-36 hrs incubation at 35°C .
- 3.2. The R-CARD® Enterococcus is ready-to-use for detecting *Enterococcus faecalis* in liquid samples.

4. Interferences

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

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® Enterococcus

8. Quality Assurance/Quality Control

8.1. Quality control

- 8.1.1. Each lot of the R-CARD® Enterococcus 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. *Enterococcus faecalis* ATCC 24212 may be used as the positive control. *Escherichia coli* ATCC 25922 may be used as the negative 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 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 15-36 hrs.

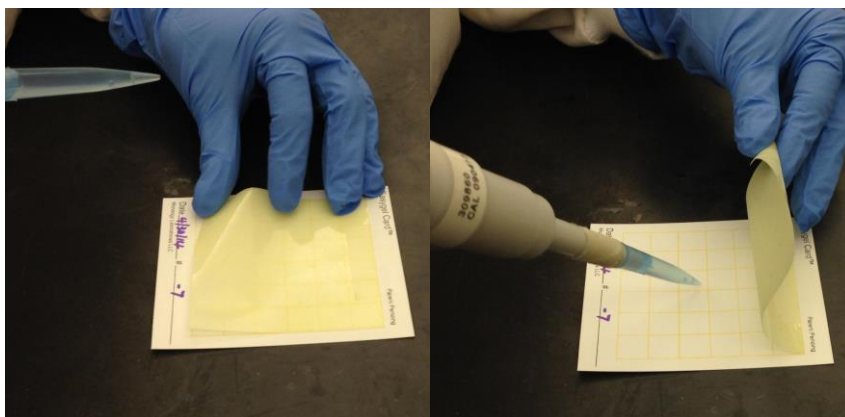


Photo 1. Open the film

Photo 2. Lift the film

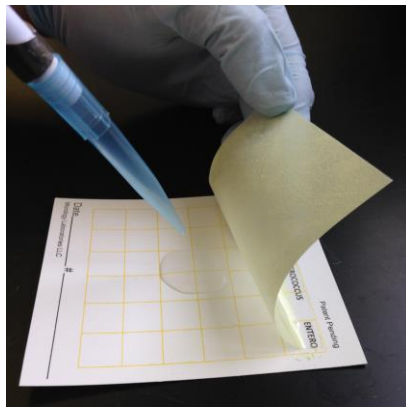


Photo 3. Pipette 1 mL sample

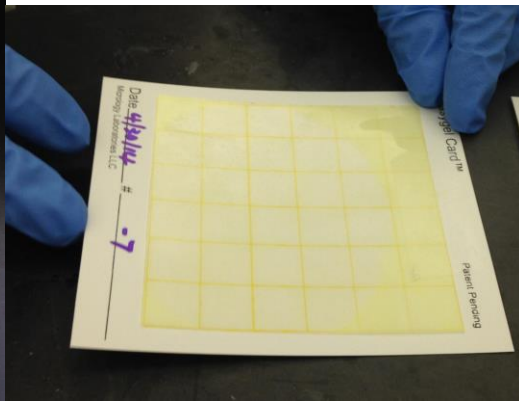


Photo 4. Cover with the top film

10. Data Analysis and Calculations

10.1. Count the number of colonies detected.

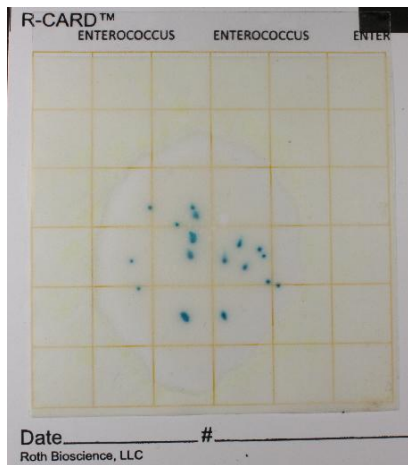


Photo 1.

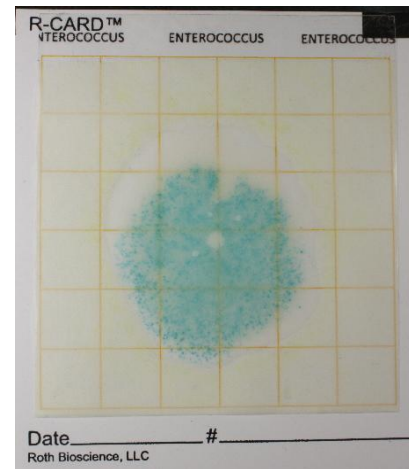


Photo 2.

Photo 1 shows 17 CFUs (colony forming units), while Photo 2 shows the appearance of TNTC (too numerous to count) where there are excessive numbers of colonies and the sample should have been diluted.

Count green/teal colonies present on the card between 15-36 hr incubation and record as the number of *Enterococcus*/volume of sample for that test.

Green, turquoise colonies are counted as *Enterococcus*.

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