



## **Standard Operating Procedure**

### **R-CARD® Enterococcus**

#### **Rapid Detection of *Enterococcus faecalis***

Roth Biosciences, LLC  
220 Bloomingdale Dr.  
Bristol, IN. 46507  
(574) 533-3351  
[hello@rothbioscience.com](mailto:hello@rothbioscience.com)  
[www.rothbioscience.com](http://www.rothbioscience.com)

## 1. Scope and Application

1.1. This method describes a procedure with the R-CARD® Enterococcus for detection and enumeration of *Enterococcus faecalis* bacterium within 15 to 36 hrs. 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. The detection limit is one colony forming unit (CFU) per sample.

1.2.

## 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 -36 hrs. 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  $35^{\circ}\text{C}$ .

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.

## 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 growth control.  
*Escherichia coli* ATCC 25922 may be 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.
- 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.
- 9.5. Cover with the film, and wait 1 min to allow liquid to spread automatically.
- 9.6. Incubate at 35±0.5°C for 15-36 hrs.

**10. Data Analysis and Calculations**

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

**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