



## **Standard Operating Procedure**

### **R-CARD® Total Count**

(Duogen™ Technology)

## **Rapid Test Method for Determining Total Microbial Count**

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## 1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD® Total Count (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of total microbe populations within 24-48 hrs. incubation time. (The incubation time and temperature depend on the type of microorganism under study.) This test can be applied to water, food or other materials.
- 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 25-35±0.5°C for 24 - 48 hrs. (The incubation time and temperature depend on the type of microorganism under study.) In ambient light, colonies will appear as light to dark red dots on the light background.

## 3. Method Definition

- 3.1. In this method, bacteria will produce pale/dark red colonies on R-CARD® Total Count
- 3.2. R-CARD® Total Count is ready-to-use for detecting microbes in liquid samples.

## 4. Interferences

- 4.1. Generally none

## 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® Total Count

## 8. Procedure

- 8.1. Prepare samples as usual and make a serial dilution if necessary.
- 8.2. Wear glove and open the top portion (film) or use sterile forceps (see photos 1-2)
- 8.3. Select dilutions of the sample to produce 20-150 colonies on the cards.
- 8.4. Pipette 1 mL of the sample on the center of the card (photo 3).
- 8.5. Cover with the film, and wait 1 min to allow liquid to spread automatically. There is no need to use a spreader. (photo 4).
- 8.6. Incubate at the preferred temperature for 24-48 hrs.

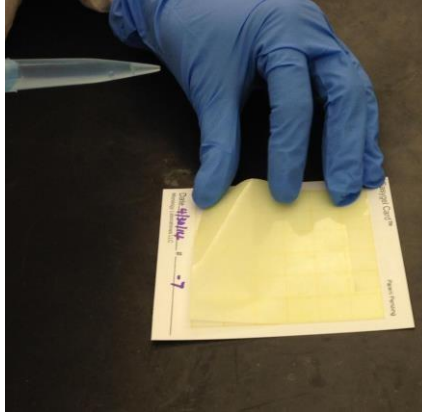


Photo 1. Open the film

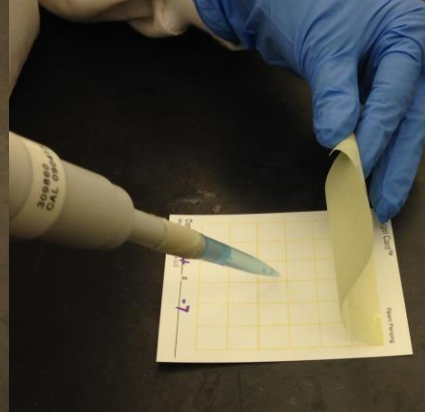


Photo 2. Lift the film

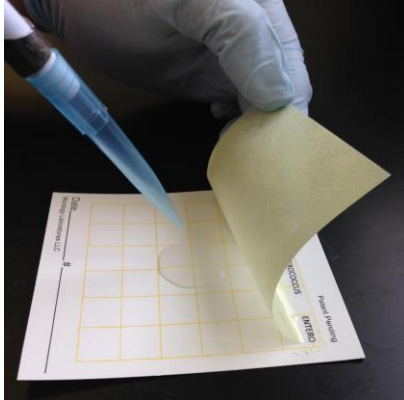


Photo 3. Pipette 1 mL sample

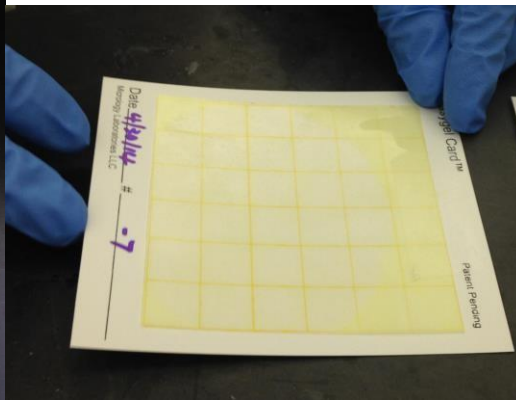


Photo 4. Cover with the top film

## 10. Data Analysis and Calculations

- 10.1. Count the number of pale/dark red colonies (Photo 5) present on the card, noting the incubation time and temperature and record as the number of CFU's/volume of sample for that test.

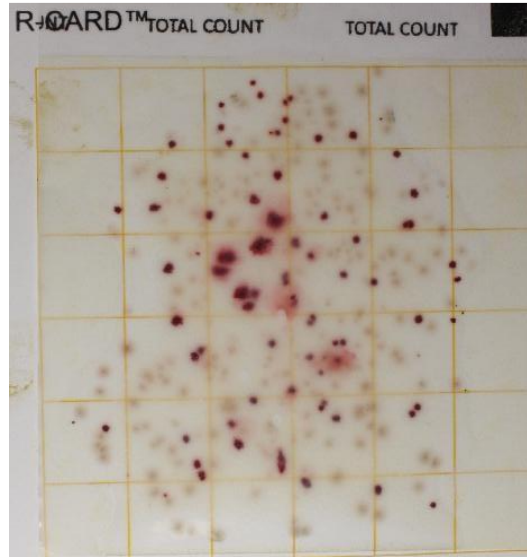


Photo 5

## 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