



## **Standard Operating Procedure**

### **R-CARD® Listeria** (Duogen™ Technology)

## **Rapid Test Method for Listeria spp.**

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## 1. **Scope and Application**

- 1.1. This method describes a procedure with the R-CARD® Listeria (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of Listeria species within 20 to 48 hrs. Because Listeria are disease causing inhabitants of the intestinal tract of warm-blooded animals, their presence in water and food samples are an indication of possible fecal pollution and indicate a danger for causing enteric disease if ingested. This test method 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 covered by the top film. The liquid sample will spread laterally automatically within 1 min. The card is then incubated at 35±0.5°C for 20-48 hr. In ambient light, green/teal colonies (CFUs) are indicative of Listeria spp.
- 2.2. R-CARD Listeria contains nutrients to assure the growth of the target organisms, buffers to maintain appropriate pH, and inhibitors to reduce growth of non-target organisms.

## 3. **Definitions**

- 3.1. In this method, Listeria species are those bacteria which produce green/teal colonies between 20-48 hr incubation, and other bacterial types either will not grow or are generally colorless.
- 3.2 R-CARD® Listeria is ready-to-use for detecting Listeria species in liquid samples.

## 4. **Interferences**

- 4.1. If the liquid sample is too turbid, it may become difficult to observe the presence of non-Listeria bacterial organisms.

## 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® Listeria

## 8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of R-CARD® Listeria 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. *Listeria monocytogenes* ATCC 3313 is used as the positive control. *Enterobacter aerogenes* ATCC 13048 or *Escherichia coli* ATCC 25922 may be used as negative growth control microorganisms..

## 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 Listeria colonies on the cards.

9.4. Pipette 1 mL of the sample on the center of the card (photo 3).

9.5. Cover the film, and wait 1 min to allow liquid to spread automatically. There is no need to use a spreader. (photo 4).

9.6. Incubate at  $35\pm 0.5^{\circ}\text{C}$  for 20-48 hrs

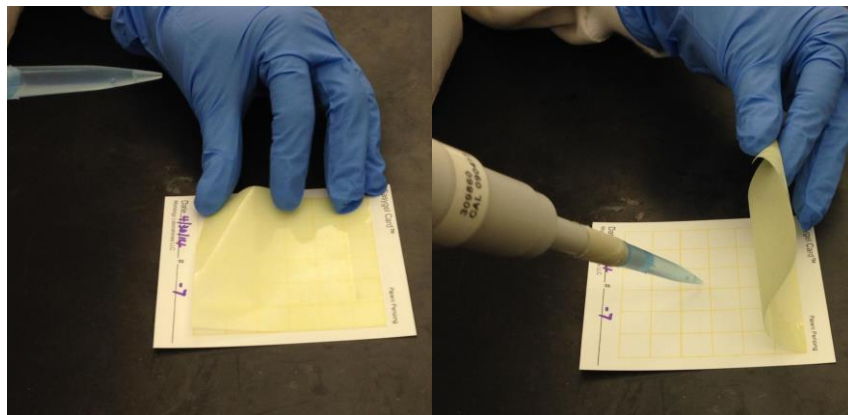


Photo 1. Open the film

Photo 2. Lift the film

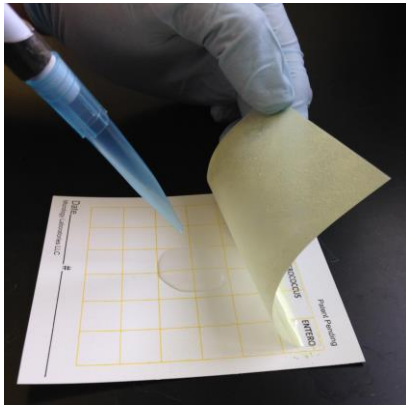


Photo 3. Pipette 1 mL sample

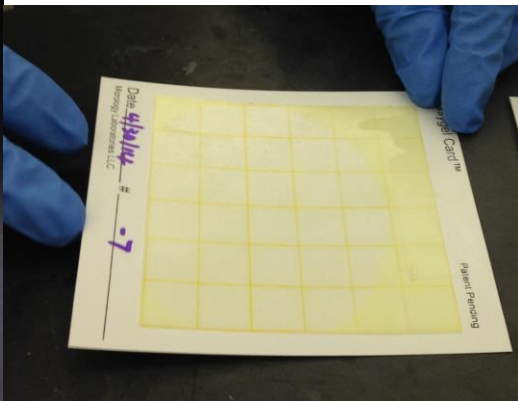
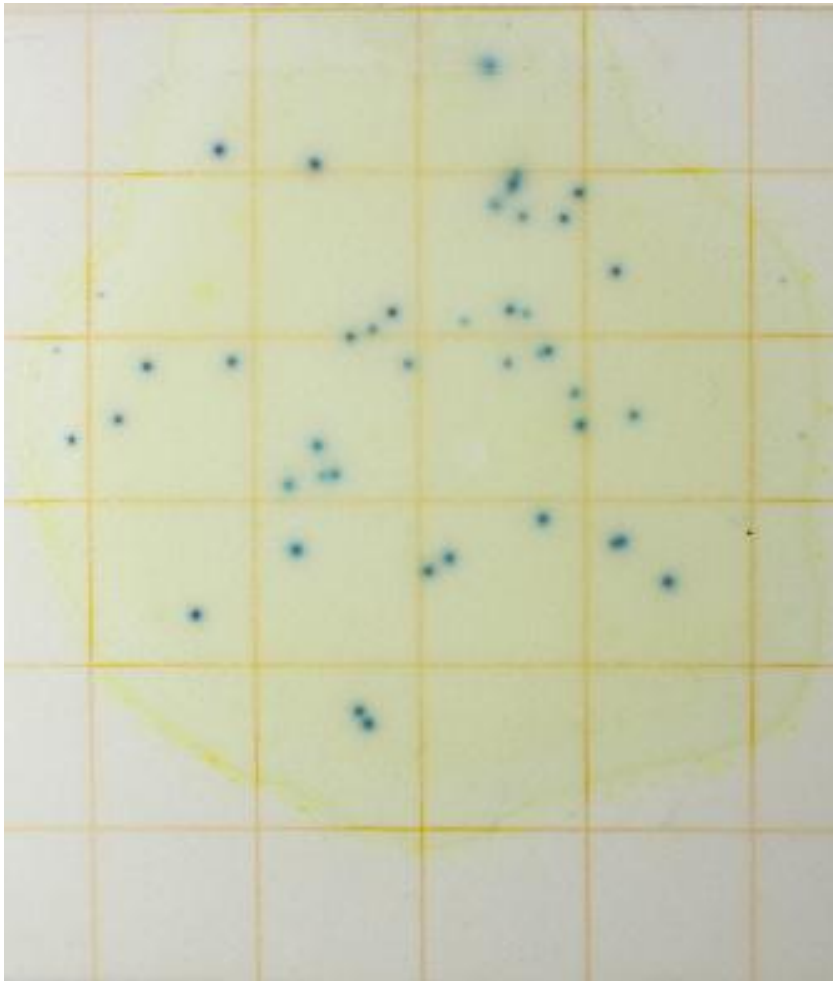


Photo 4. Cover the film

## 10. Data Analysis and Calculations

- 10.1. Count the number of green/teal colonies detected on the card between 20-48 hr incubation and record as the number of Listeria/volume of sample for that test.



Green/teal colonies are counted as Listeria

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.