



Standard Operating Procedure

R-CARD® Lactic Acid Bacteria

Rapid Test Method for *Lactobacillus* spp.

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1. Scope and Application

- 1.1. This method describes a procedure with the R-CARD® Lactic Acid Bacteria (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of *Lactobacillus* spp. within 24 to 48 hrs. *Lactobacillus* are commonly found in, or added to, fermented food products and are common constituents of Probiotic formulations used to normalize the gut flora and eliminate unwanted constituents of the gut flora. Tests to identify the presence of these bacteria or to measure the levels in foods are very useful in treating abnormalities in the gut microflora. This test method can be applied to food, water, 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 24-48 hrs. In ambient light, green/teal colonies (CFUs) are indicative of *Lactobacillus* spp..
- 2.2. R-CARD® Lactic Acid Bacteria 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, *Lactobacillus* are those bacteria which produce green/teal colonies between 24-48 hr incubation, and other bacterial types either will not grow or are generally colorless.
- 3.2 R-CARD® Lactic Acid Bacteria ready-to-use for detecting *Lactobacillus* in liquid samples.

4. Interferences

- 4.1. If the liquid sample is too turbid, it may become difficult to observe the presence of non-Lactic Acid 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® Lactic Acid Bacteria

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of R-CARD™ Lactic Acid Bacteria 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. 8.1.2 *Lactobacillus* sp. 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 Lactic Acid bacterial 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±0.5°C for 24-48 hrs . .

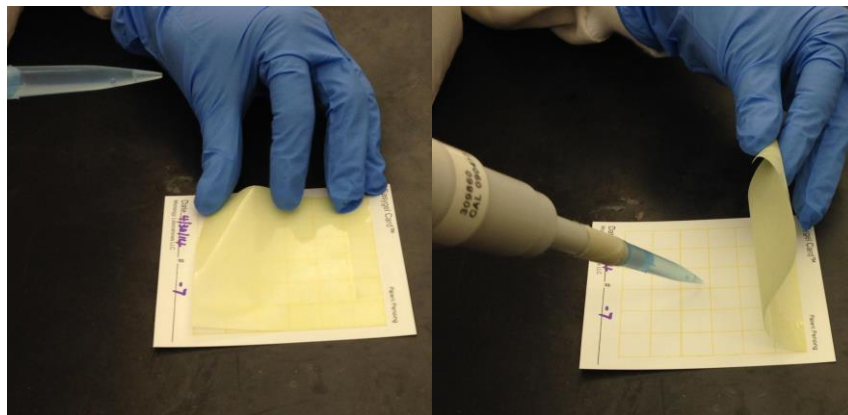


Photo 1. Open the film

Photo 2. Lift the film

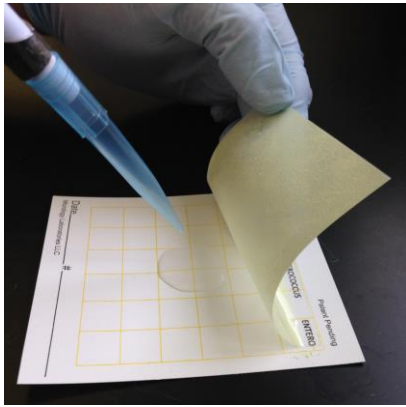


Photo 3. Pipette 1 mL sample

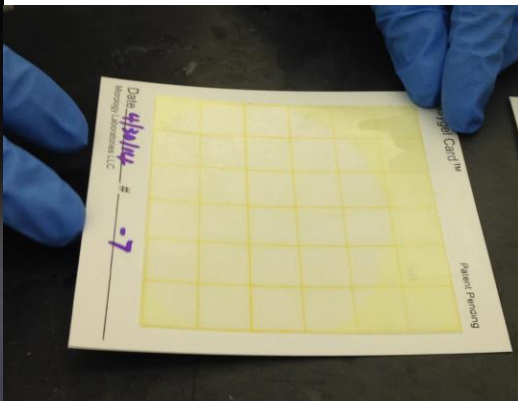
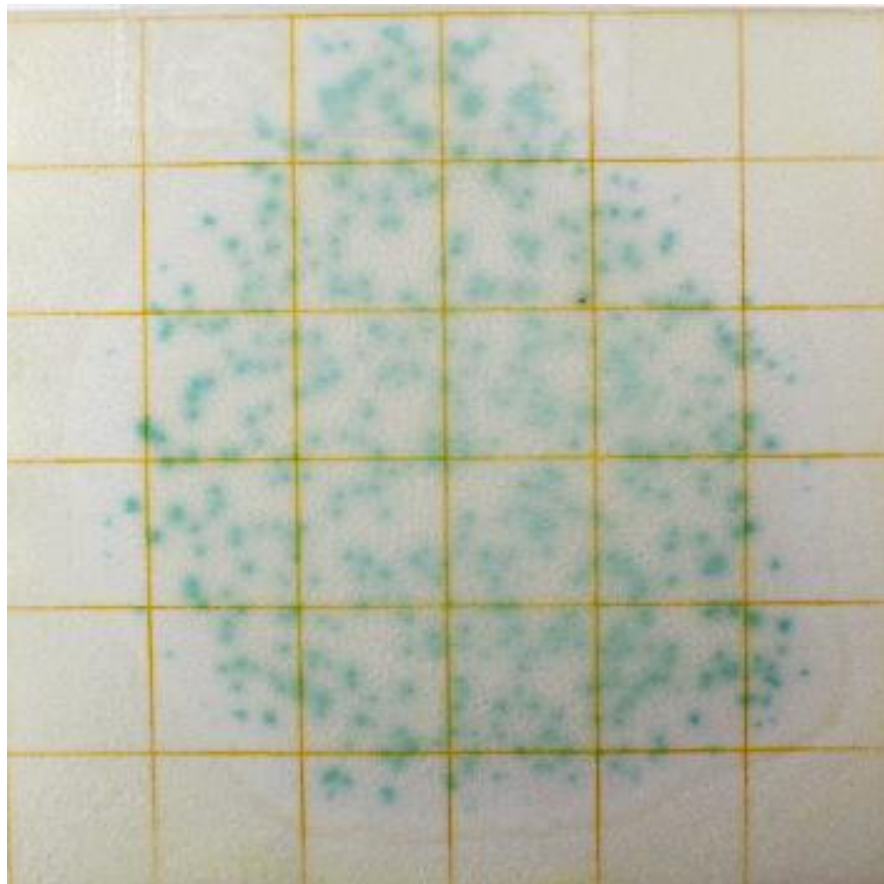


Photo 4. Cover the film

10. Data Analysis and Calculations

- 10.1. Count the number of green/teal colonies detected on the card between 24-48 hr incubation and record as the number of Lactic Acid bacteria/volume of sample for that test.



Green/teal colonies are identified and counted as Lactic Acid Bacteria

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.