Standard Operating Procedure

R-CARD™ Orange Serum

Rapid Test Method for Citrus Related Bacteria

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1. Scope and Application

1.1. This method describes a procedure with the R-CARD™ Orange Serum (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of Citrus related spoilage bacteria and yeasts and molds within 24 to 72 hrs incubation time at a predetermined temperature (generally 25-35^{0} C). Lactic Acid bacteria (*Lactobacillus spp.* and related types*), yeasts, and molds* are commonly found in/on Citrus products and may be involved in product rotting or spoilage. R-CARD Orange Serum tests identify the presence of these microbes or to measure the levels in citrus fruit or products can be very useful in treating and remediating these conditions. This test method can be applied to solid, aqueous, or other product variations.

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 the predetermined temperature (commonly 25-35°C for 24-72 hrs. In ambient light, green/teal colonies (CFUs) are indicative of Citric related microbes.

2.2. R-CARD Orange Serum formula 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, citric product related microbes are those which produce green/teal colonies within the chosen incubation period, and other microbial types either will not grow or are generally colorless.

3.2 R-CARD Orange Serum is ready-to-use for detecting Citrus related microbes in liquid samples.

4. Interferences

4.1. If the liquid sample is too turbid, it may become difficult to observe the presence of non-Citrus related 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™ Orange Serum

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of R-CARD™ Orange Serum 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 Citrus related 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 25-35°C for 24-48 hrs.

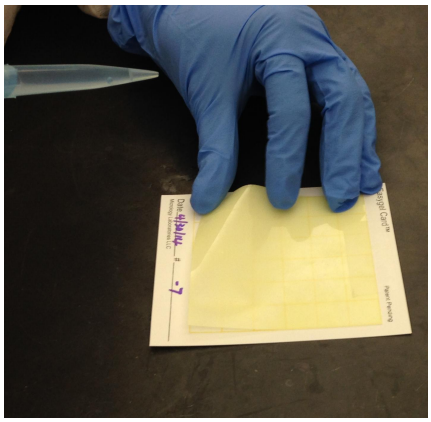


Photo 1. Open the film

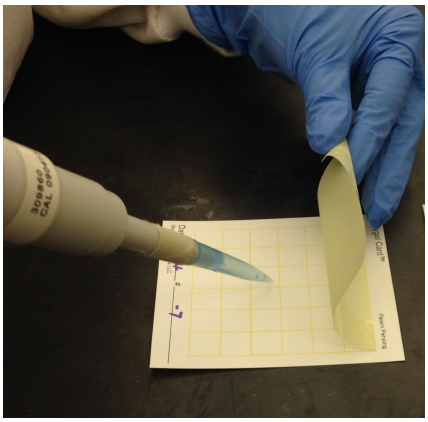


Photo 2. Lift the film

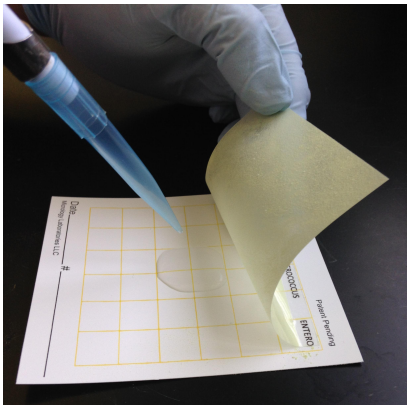


Photo 3. Pipette 1 mL sample

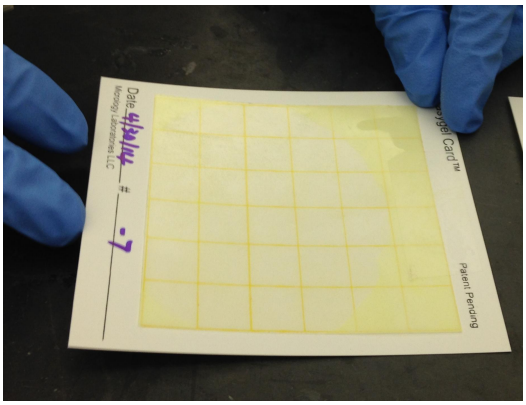
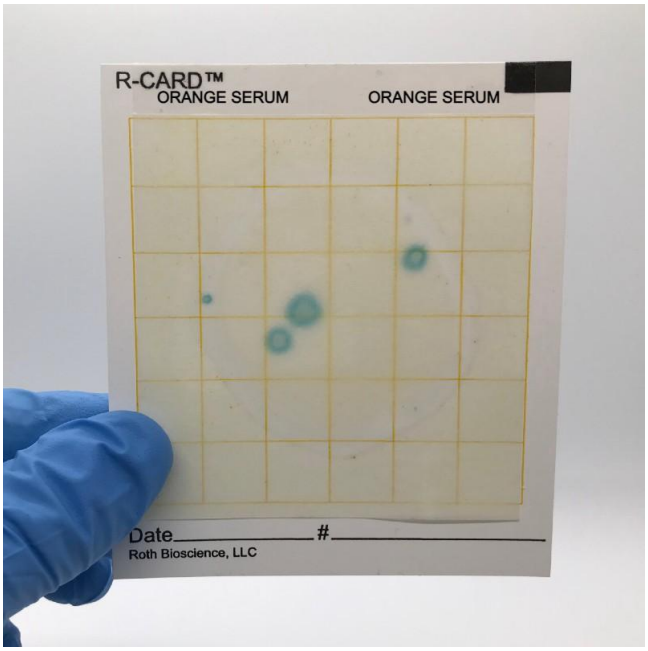


Photo 4. Cover the film

10. Data Analysis and Calculations

10.1. Count the number of green/teal colonies detected on the card between the chosen incubation time and temperature and record as the number of Citrus related microbes/volume of sample for that test.



11. Pollution Prevention and Waste Management

11.1. All biohazardous waste should be sterilized at 121ºC for 30 min prior to disposal. Laboratory personnel should use pollution control techniques to minimize waste generation wherever possible.