Standard Operating Procedure

R-CARD® Salmonella EC

Rapid Test Method for Salmonella spp. and E. coli

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1. Scope and Application

1.1. This method describes a procedure with the R-CARD® Salmonella EC (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of Salmonella species *and E. coli* together on one R-CARD® within 18 to 24 hrs. Because Salmonella are common 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. Also, E. coli is a well-known bacterium used in many test methods as a target organism whose presence verifies contamination of the test material by fecal material. This test method can be applied to water, food or other materials. The ability to detect either or both of these organisms by a single test simplifies work load and saves time and cost of testing for each by a separate procedure.

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 18 -24 hr. In ambient light, black colonies (CFUs) are indicative of Salmonella spp. and green colonies are indicative of *Escherichia coli*.

2.2. R-CARD® Salmonella EC 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, Salmonella species are those bacteria which produce black colonies between 18-24 hr incubation, *E. coli* grow as green colonies, and other bacterial types either will not grow, yellow colonies without black center, or are generally colorless.

3.2 R-CARD® Salmonella EC is ready-to-use for detecting Salmonella species and *E. coli* in liquid samples.

4. Interferences

4.1. If the liquid sample is too turbid, it may become difficult to observe the presence of non-Salmonella or *E. coli* 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® Salmonella EC

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of R-CARD® Salmonella EC 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. 8.1.2 *Salmonella typhimurium* ATCC 38387 and E.coli ATCC 25922 is used as the positive control. *Enterobacter aerogenes ATCC 13048* or *Pseudomonas aeruginosa* ATCC 10145 or 27853 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 Salmonella/*E. coli* 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 18-20 hrs (no more 24 hrs).

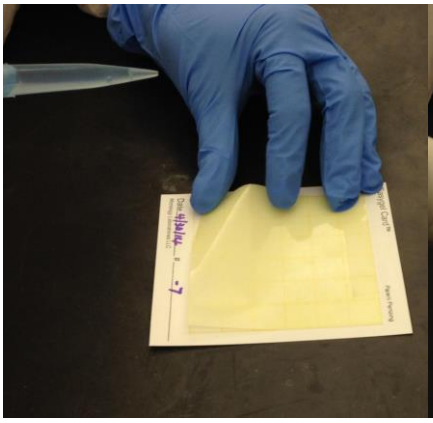


Photo 1. Open the film

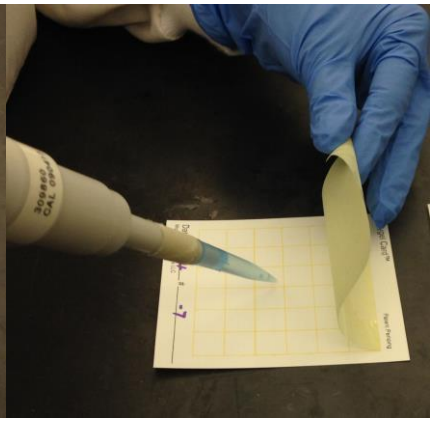


Photo 2. Lift the film

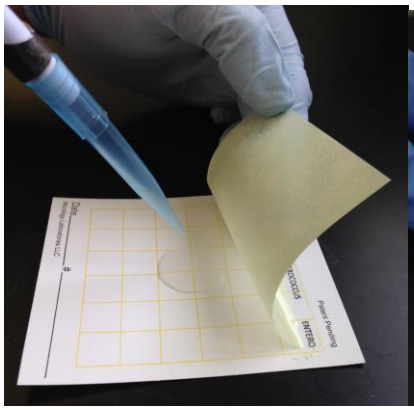


Photo 3. Pipette 1 mL sample

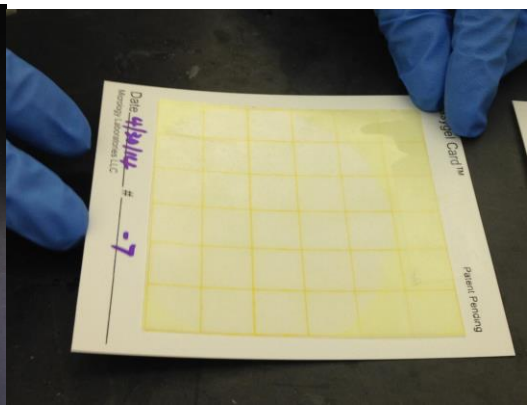
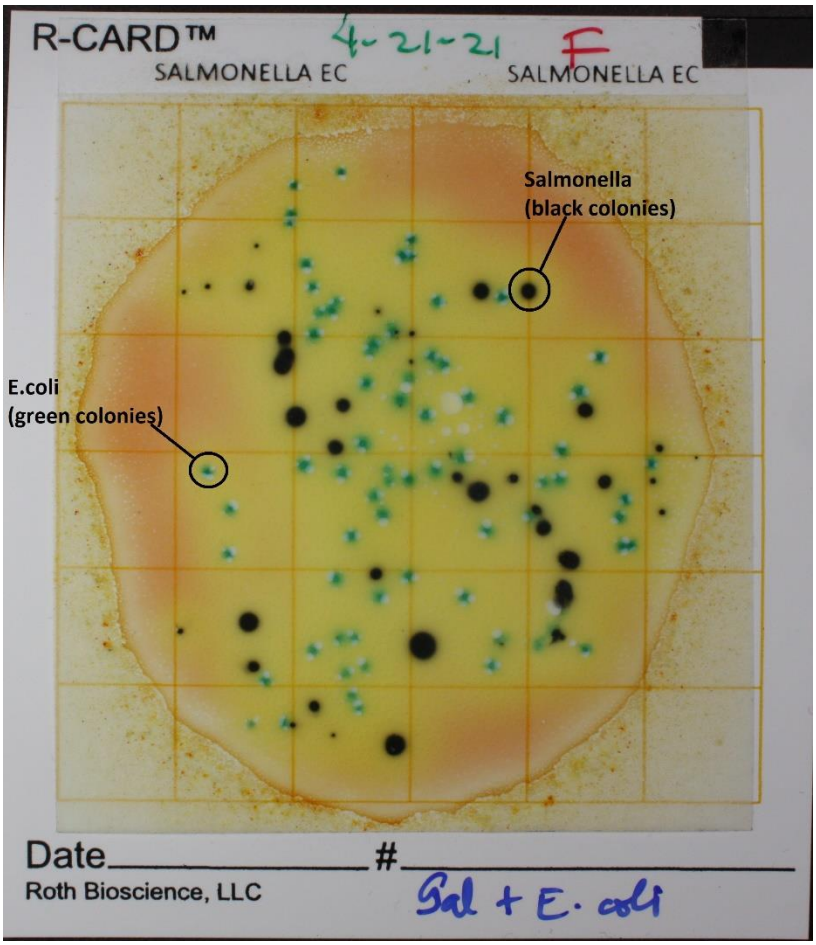


Photo 4. Cover the film

9.7. Count the number of black colonies detected on the card between 18-24 hr incubation and record as the number of Salmonella/volume of sample for that test and the number of green colonies indicates the number of *E. coli*/volume of that test sample.



Black colonies are counted as Salmonella and green colonies are *E. coli*

10. **Pollution Prevention and Waste Management**

10.1. 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.