Standard Operating Procedure

R-CARD® ECC-A

(Duogen® Technology)

Rapid Test Method for *E. coli* and Coliform

(eliminating *Aeromonas* false positive as Coliform)

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1. Scope and Application

1.1. This method describes a procedure with the R-CARD® ECC-A (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of *Escherichia coli* (*E. coli*) and total coliforms (eliminating Aeromonas false positive as Coliform) within 15 to 20 hrs. Because E. coli are natural inhabitants of the intestinal tract of warm-blooded animals, their presence in water and food samples are an indication of fecal pollution and the possible presence of other enteric pathogens. 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 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 15 -20 hr. In ambient light, blue to dark purple and dark pink/red colonies are counted as *E.coli* and coliforms, respectively, and *Aeromonas species, if* present, are virtually undetectable as they grow as small, light yellow colonies that are very difficult to see on the R-CARD® background. The best way to determine if Aeromonads are present is to do a side by side comparison of the test sample on one R-CARD® ECC-A and one R-CARD® ECC and count the Coliform results. If there are more dark pink/red CFUs on the standard R-CARD® ECC test card than on the R-CARD® ECC-A, the differential indicates the presence and numbers of Aeromonads in the sample.

2.2. The R-CARD® ECC-A 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, *E. coli* are those bacteria which produce dark blue to purple colonies and other coliforms will produce light to dark pink/red colonies on R-CARD® ECC-A between 15-20 hr incubation. Aeromonads will grow, but are generally imperceptible as they appear as very light yellow CFUs that do not contrast with the R-CARD® background (see 2.1 explanation).

3.2 R-CARD® ECC-A is ready-to-use for detecting E. coli and coliform (absent coliform false positive as Aeromonas spp.) in liquid samples.

4. Interferences

4.1. If the liquid sample is too turbid, it may become difficult to observe light colored colonies.

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® ECC-A

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of R-CARD® ECC-A 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 *E. coli* ATCC #11775 or 25922 is used as the positive control. *Enterobacter aerogenes ATCC 13048* is used as the coliform positive control, and *Pseudomonas aeruginosa* ATCC 10145 or 27853 is used.as the negative growth control microorganism.

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 coliform/*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 15-20 hrs (no more 24 hrs). Incubation may be at 44.5±0.2°C for detecting ***E. coli* only**.

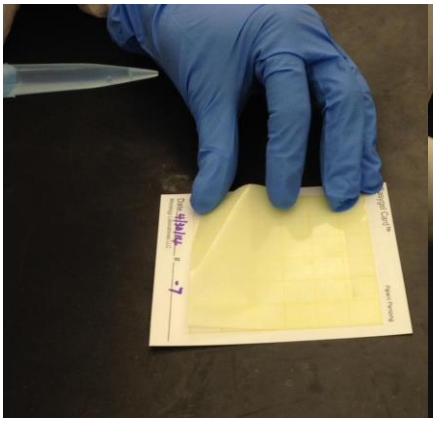

Photo 1. Open the film

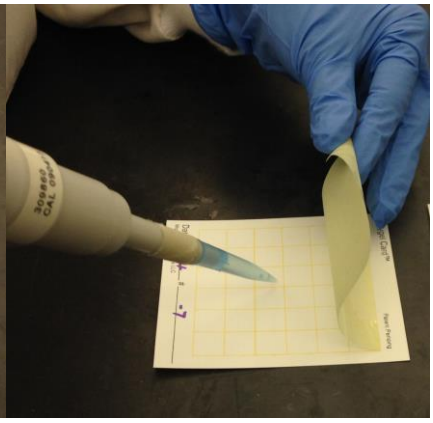

Photo 2. Lift the film

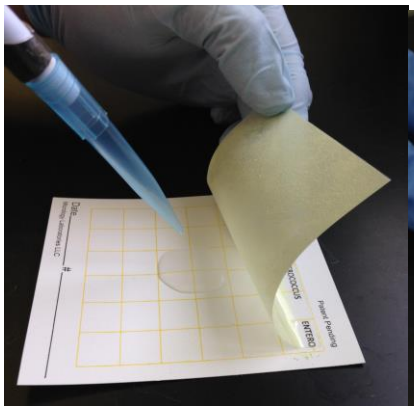

Photo 3. Pipette 1 mL sample

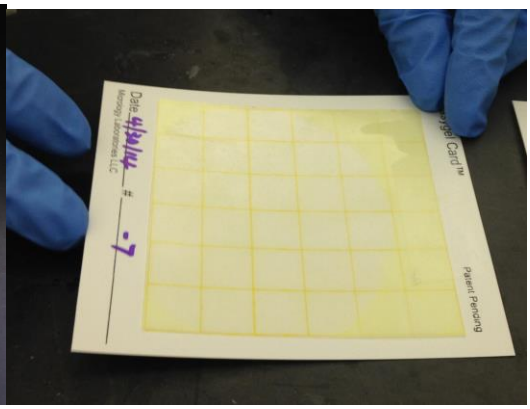

Photo 4. Cover the film

10. Data Analysis and Calculations

10.1. Count the number of colonies detected by dark blue to purple or light to dark pink/red colonies present on the card between 15-20 hr incubation and record as the number of *E. coli*/coliform/volume of sample for that test.

Dark blue to purple colonies for *E. coli* and light to dark pink/red colonies for other coliforms. Aeromonas species are very light yellow and generally unobservable due to the R-CARD® background color.

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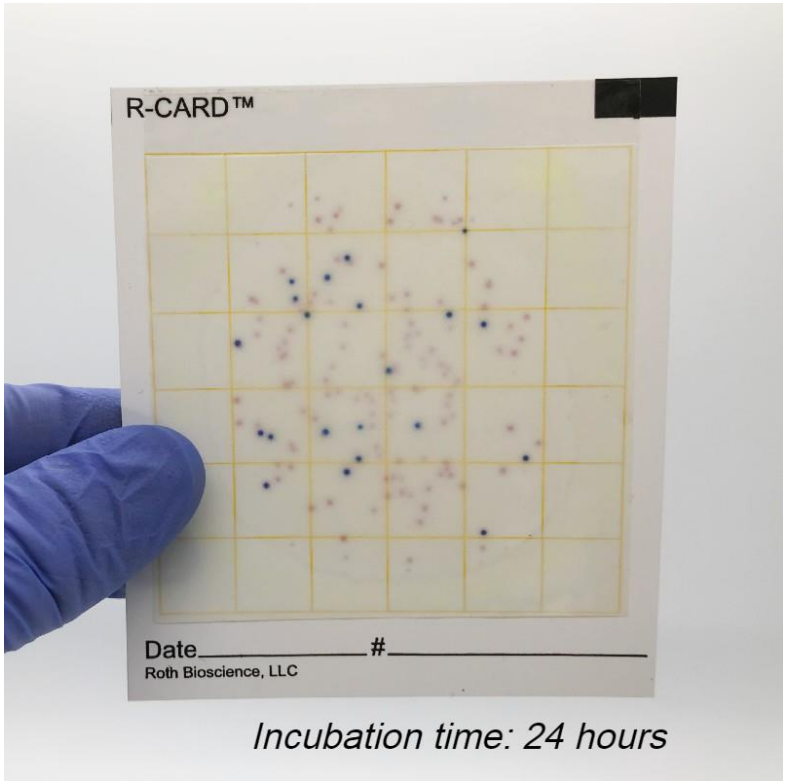

11. **Pollution Prevention and Waste Management**

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.