Standard Operating Procedure

R-CARD® KwikCount® EC

Rapid Test Method for *Escherichia coli* (*E. coli*) In Ambient Waters

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1. Scope and Application

1.1. This method describes a procedure with the R-CARD® KwikCount® EC (Roth Bioscience, LLC) for a rapid detection and enumeration of *Escherichia coli* (*E. coli*) within 7 to 9 hrs. Because these bacteria are natural inhabitants of the intestinal tract of warm-blooded animals, their presence in water samples are an indication of fecal pollution and the possible presence of other enteric pathogens. This test for E. coli can be applied to fresh water, or other waters matrices. The R-CARD® KwikCount® EC medium (Roth Bioscience LLC, Goshen, Indiana) can be used to detect and enumerate E. coli within 7 to 24 hrs.

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 41±0.5°C for 7 -9 hr. The R-CARD® are then viewed in a dark place using a long wave UV light (366nm preferred) and any fluorescent colony is counted as E.coli .

2.2. R-CARD® KwikCount® EC contains nutrients that promote the growth of the target organisms and buffers to maintain appropriate pH. It also contains a fluorogenic/chromogenic enzyme substrate mixture that is used to cause E.coli to fluoresce and is detectable as early as 7 hrs incubation time (7-9 hrs incubation is the peak fluorescent intensity.) The fluorescent light will gradually decrease until about 24 hrs when it will be totally dissipated. Beginning about 14-16 hrs incubation, the E.coli colonies will not only fluoresce in UV light, but will also appear as blue/purple colonies in ambient light. This phenomenon of a coloring compound having two unique properties simultaneously has been trademarked as a DUOGEN®.

2.3. The R-CARD® KwikCount® EC method therefore incorporates and includes the advantages of utilizing a Duogen®. When the blue fluorescence has dissipated, the E.coli colonies will continue to be present as blue/purple colonies that may continue to grow and intensify in color. The blue/purple colonies should not be counted after 48 hrs incubation.

3. Method Definition

3.1. In the R-CARD® KwikCount® EC method, ***E. coli*** are those bacteria which produce fluorescent colonies on the medium between 7-16 hrs. incubation when using a long wave UV light source (366nm preferred).

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

4. Interferences

4.1. In the case that excessive crowding is observed on the R-CARD®, samples should be recollected and rerun at a higher dilution. Very small colored colonies should not be counted unless they are isolated as individual colonies with densities below 80 CFU per CARD.

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. Suggested Equipment and Supplies

6.1. Sterile pipettes (1 to 25 mL)

6.2. Forceps: smooth, flat, sterilizable metal forceps.

6.3. Microscope: 10 to 15 X magnification binocular wide-field dissecting microscope.

6.4. Light box

6.5. Bunsen burner or alcohol lamp for sterilizing forceps..

7. Reagents and Standards

7.1. Sterile deionized or distilled water

7.2. R-CARD® KwikCount® EC

8. Quality Assurance/Quality Control

8.1. Quality control

8.1.1. Each lot of the R-CARD® KwikCount® EC medium should be evaluated by the laboratory by preparing three plates of the medium (one to serve as an un-inoculated control, one to serve as a negative growth control, and one to serve as positive control).

8.1.2. *E. coli* ATCC #11775 or 25922 is used as the positive control. *Klebsiella pneumoniae ATCC 31488* is used as the negative control, and also *Pseudomonas aeruginosa* ATCC 10145 or 27853 may be used.as a negative growth control microorganism.

9. Procedure

9.1. Prepare samples as usual and make a serial dilution if necessary.

9.2. Wear glove and lift the top portion (film) or use sterile forceps (see photos 1-2)

9.3. Select dilutions of the sample to produce 20-150 *E. coli* colonies on the cards.

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

9.5. Cover with the film, and wait about1-2 min to allow liquid to spread automatically. There is no need to use a spreader. (photo 4).(Some samples do not automatically spread in as large an area as may be wanted, but it is a simple matter to encourage spreading by gently applying pressure on the top after it is lowered on the inoculum.

9.6. Incubate at 41±0.5°C for 7-24 hrs (no more than 48 hrs).

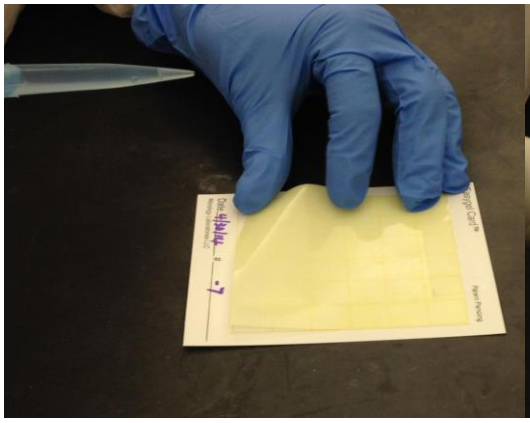


Photo 1. Open the film

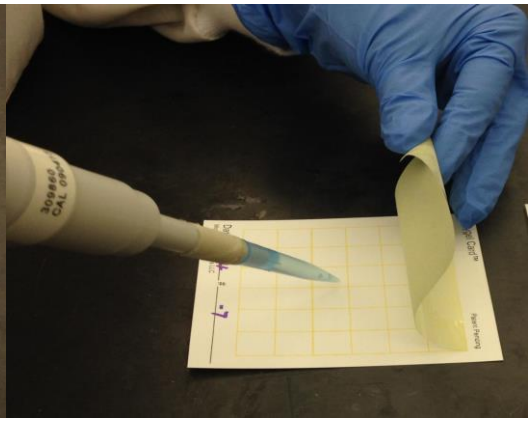


Photo 2. Lift the film

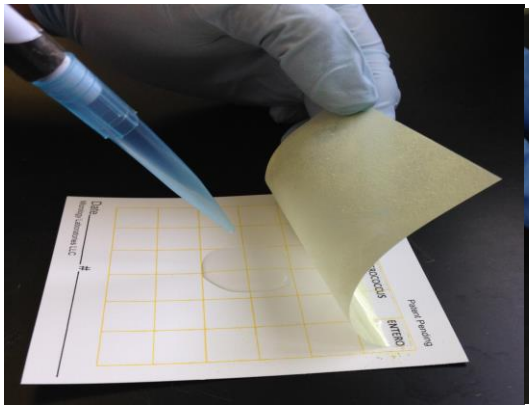


Photo 3. Pipette 1 mL sample

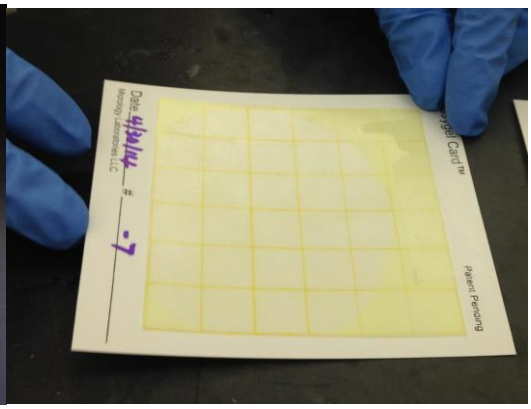
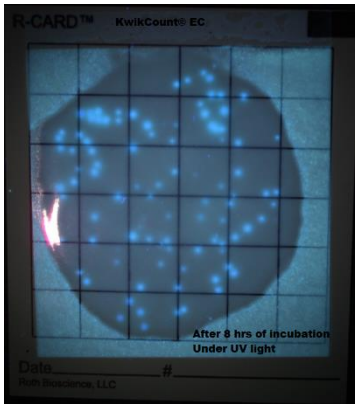


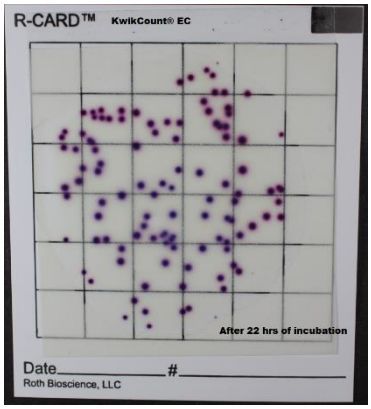
Photo 4. Cover with the top film

10. Data Analysis and Calculations

10.1. Count the number of colonies detected by blue fluorescence present on the card between 8-16 hr incubation and record as the number of E. coli / volume of sample for that test.



UV light 8 hr
Blue fluorescence (Duogen® effect) E.coli



Ambient light at 20 hr
Blue-purple (Chromogenic color) E.coli

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.