



Standard Operating Procedure

R-CARD® Yeast and Mold

Rapid Test Method for Detecting Yeast and Mold In The Air or On/In Surfaces

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1. Scope and Application

1.1. This method describes a procedure with the R-CARD® Yeast and Mold (Roth Bioscience, LLC, Goshen, Indiana) for detection and enumeration of Molds and Yeast within 24-72 hrs. Molds or yeasts or their spores are present in virtually every environmental setting on the face of the earth if special care is not exerted to eliminate them. They are responsible for many types of illnesses in humans, plants, and other animals, including allergic reactions, food poisoning, pulmonary and intestinal diseases. Anywhere that provides water and nutrients will support their establishment and growth. This describes two methods for determining, the prevalence of mold spores such as **(#1)** in the air in an open space such as a laboratory or food production working area, or in your kitchen, living room, or bedroom, and **(#2)** a second method for determining mold on the surface(s) of solid structures such as floors, tables, etc.

1.2 The detection limit is one colony forming unit (CFU) per sample.

2. Summary of Methods

2.1. **(#1) In The Air** - Lift the top piece of the R-CARD®. 0.50 mL sterile water is pipetted on the center of the R-Card and releasing the top piece down and wait for 2 minutes for it to gel. Lift the top piece and hold it open using a small clip. Mold spores settle from the air during the chosen exposure time, and adhere on the gelled medium. After the predetermined exposure time, 1 mL of sterile water is pipetted to the center of the card and the top piece is loosened and allowed to flop over the liquid. The liquid will spread laterally automatically within 1 min. The card is then incubated at 25-30°C for 24-72 hr. In ambient light, green/teal colonies (CFUs) are indicative of the target organisms.

2.2. **(#2) On/In Surfaces** - A liquid sample is pipetted on the center of the card, and covered by the top film or sterile water may be pipetted on the center of the card and the sample may be stirred into the water after collection on a sterile swab moistened with sterile water. The liquid sample will spread laterally automatically within 1 min. The card is then incubated at 25-30°C for 24-72 hr. In ambient light, green/teal colonies (CFUs) are indicative of the target organisms. Molds form circular spreading colonies and yeasts are more compact and non-spreading.

2.3. R-CARD Yeast and Mold 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, molds produce green/teal colonies between 24-72 hr incubation, and other microbes generally are inhibited and will not grow.

3.2 R-CARD® Yeast and Mold is ready-to-use for detecting mold spores in the air.

4. Interferences

4.1 Generally absent

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. Small clip
- 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® Yeast and Mold

8. Quality Assurance/Quality Control

- 8.1. Quality control
 - 8.1.1. Each lot of R-CARD® Yeast and Mold 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. *Aspergillus* or *Penicillium* spp. 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 for (#1)) In The Air

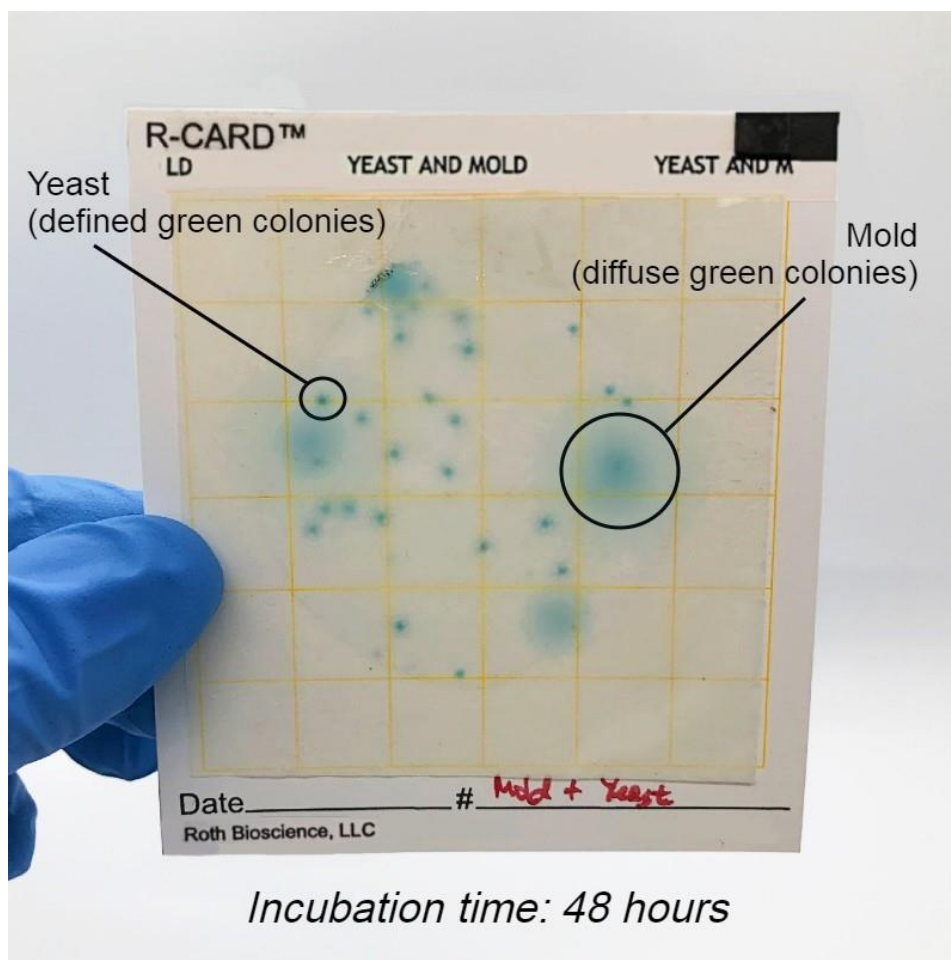
- 9.1. Lay the R-Card® on a flat surface and open the top piece (film).
- 9.2. Pipette 0.5 mL of sterile water
- 9.3. Release top piece (film) down to cover the liquid and wait for 2 minutes for it to gel
- 9.4. After 2 minutes, lift the top piece (film) again and hold it open using a small clip so that the gelled medium is exposed to air
- 9.5. Pipette 1 mL of sterile water on the center of the card after exposure time is done
- 9.6. Loosen the top piece and allow to flop over the liquid. The liquid will spread laterally automatically within 1 min
- 9.7. Incubate at 25-30°C for 24-72 hrs

10. Procedure for (#2)) On/In Surfaces

- 10.1. Prepare samples as usual and make a serial dilution if necessary
- 10.2. Wear glove and open the top portion (film) or use sterile forceps
- 10.3. Select dilutions of the sample to produce 20-75 Mold or Yeast colonies on the cards.
- 10.4. Cover the film, and wait 1 min to allow liquid to spread automatically. There is no need to use a spreader.
- 10.5. Incubate at 25-30°C for 24-72 hrs

11. Data Analysis and Calculations

- 11.1. Count the number of green/teal colonies detected on the card between 24-72 hrs incubation and record as the number of Mold or Yeast/volume of sample for that test.



Green/teal colonies are counted as Mold (25 C./48 hrs incubation)

12. **Pollution Prevention and Waste Management**

- 12.1. 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.