



Standard Operating Procedure

R-CARD® Yeast and Mold

Rapid Test Method for Detecting Yeast and Mold

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INTRODUCTION:

Molds and their relatives constitute the largest body of living organisms that inhabit the earth's crust. Many have two phases, (1) a fruiting body (such as various mushrooms) that produces some type of spores that may grow into a spreading mycelium (mold) that may grow uncontrolled over moist, nutrient containing surfaces such as basement walls, window sills, etc., in houses and other places. There is a large industry built around collecting or growing edible mushrooms that are sold in grocery stores that constitute their own food group.

There is also a large industry built around the elimination of molds (2--not from mushrooms) in buildings that may produce severe allergies and disease such as *Penicillium, Aspergillus, and Stachybotrys*, **(the black mold that was responsible for the current huge commercial interest in home contamination).**

The Roth Bioscience Mold test is made to use in the finding and identifying of various mold types so that proper remediation procedures can restore a safe environment for persons in their home or work place. The accurate identification and effective remediation of molds has attracted a large group of "businesses" that may not provide accurate results or identification from their tests. **Mold spores can be found virtually in every building or home, most of which may not constitute an abnormal environment or are causing any notable problems, so users of Commercial Mold tests should check the credentials of the test and remediator provider. Many "remediators" offer free testing because they know that they can find mold, and they then convince their client that they need their expensive "remediation" protocol.**

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 Method

2.1. (#1) In The Air

Lift the top piece of the R-CARD®. 2 mL sterile water is pipetted to the circular pad on the bottom piece of the R-CARD®. Mold spores settle from the air during the chosen exposure time, and adhere on the moistened absorbent pad. After the predetermined exposure time, 1 mL of sterile water is pipetted to the pad and the top piece is loosened and allowed to flop over onto the wet pad. 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 and 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 consists of a bottom part that holds a 70 mm circular water-absorbent filter pad (which contains no other materials such as nutrients or toxins), and a top part (attached to one edge of the bottom part) which contains a powder layer of the ingredients (the specific proprietary formulation) such as nutrients, dyes, and inhibitors to eliminate growth of non-target organisms.

3. Definitions

- 3.1. In this method, molds produce green/teal colonies between 24-72 hours. incubation. Some yeasts and molds may start to appear faint at 24 hours. Incubating R-CARD® Yeast and Mold until 72 hours will more accurate results and enhanced interpretation. 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. Tape or a heavy object to hold the top piece (film) open
- 6.3. Forceps: smooth, flat, sterilizable metal forceps
- 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

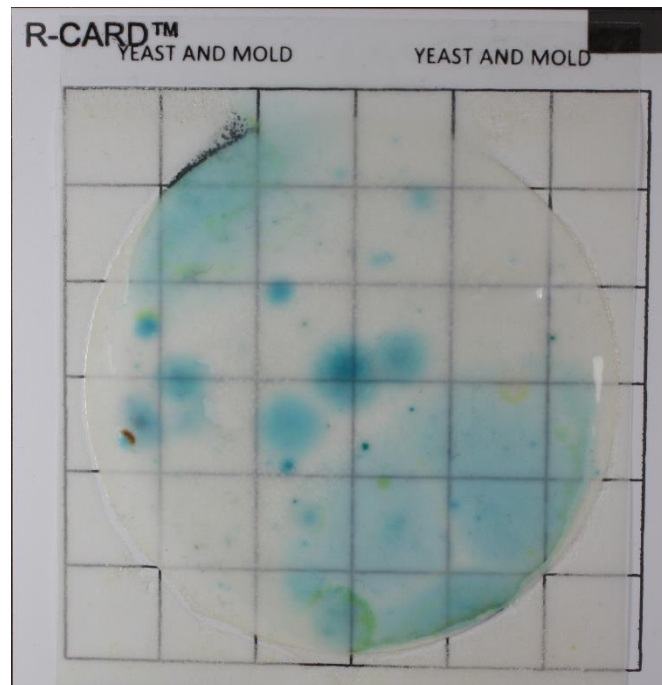
- 9.1. Place the card on a level surface, and open the top piece (film). Use a tape to keep the film open, or place a heavy object to keep the film open.
- 9.2. Pipette 2 mL of sterile water on the pad. Let it stand for chosen exposure time.
- 9.3. Pipette 1 mL of sterile water on the pad after exposure time is done.
- 9.4. Lower the top piece (film) down onto wet pad. Wait 1 minute to allow liquid to spread in a circular pattern.
- 9.5. 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. /72 hrs. incubation)

12. Pollution Prevention and Waste Management

- 9.6. 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.