# Simplate® *Yeast & Mold* Color Indicator

AOAC Official Method 2002.11
MicroVal Certificate No. 2009LR25

## Introduction

Simplate® for Yeast and Mold Color Indicator (Y&M-CI) method is used for the detection and quantification of yeast and mold in foods. It is based on Binary Detection Technology (BDT) which equates the presence of yeast and mold to the presence of a color change in the medium. The medium/sample mixture is dispensed into a Simplate® device, and incubated for a minimum of 56 h. The medium changes color in the presence of yeast and/or mold. The yeast and mold count is determined by counting the wells with changed color and referring to the Simplate® Conversion Table. The Simplate® device is packaged separately.

## Single Test Medium

### Kit Components

100 individually-packaged dehydrated Y&M-CI medium containers.

### A. Sample Preparation

**a.** Weigh 50 g of sample into 450 mL of sterile diluent [0.1% peptone water (FDA BAM Method, ISO method)]. This is a 10 – fold dilution. Masticate to homogenize.

**b.** If an alternate sample size is specified in your testing procedure or standard, prepare a 10% weight to volume suspension. For ISO method, perform initial suspensions according to specified ISO 6887 standard.

**c.** If necessary, prepare 10 – fold serial dilutions appropriate for the anticipated population of the sample.

### B. Test Procedure

**For 1.0 mL sample size**

**a.** Resuspend powdered medium with 9.0 mL of sterile deionized water containing 1 mL of Supplement A per 100 mL. Add 1.0 mL of sample and mix well. DO NOT count this reconstitu-tion as a dilution.

**For 0.1 mL sample size**

**b.** Resuspend powdered medium with 9.9 mL of sterile deionized water containing 1 mL of Supplement A per 100 mL. Add 0.1 mL of sample and mix well. This is an additional 10 – fold dilution.

## Multiple Test Medium

### Kit Components

50 multi-test dehydrated Y&M-CI medium containers. Each container is sufficient for 10 tests.

### A. Sample Preparation

**a.** Weigh 50 g of sample into 450 mL of sterile diluent [0.1% peptone water (FDA BAM Method, ISO Method)]. This is a 10 – fold dilution. Masticate to homogenize.

**b.** If an alternate sample size is specified in your testing procedure or standard, prepare a 10% weight to volume suspension. For ISO method, perform initial suspensions according to specified ISO 6887 standard.

**c.** If necessary, prepare 10 – fold serial dilutions appropriate for the anticipated population of the sample.

### B. Test Procedure

**a.** Empty contents of one container into 100 mL of sterile deionized water containing 1 mL of Supplement A per 100 mL. Shake to completely dissolve.

**NOTE:** For raw meat and spices, add 1.0 mL of Supplement M to the hydrated medium. For undiluted fruit juice containing Vitamin C or dry pet food, add 1.0 mL of Supplement V. For foods containing spreading mold, add 1.0 mL of Supplement D. Supplements are available from BioControl.

**Note:** For raw meat and spices, add 0.1 mL of Supplement M to the hydrated medium. For undiluted fruit juice containing Vitamin C or dry pet food, add 0.1 mL of Supplement V. For foods containing spreading mold, add 0.1 mL of Supplement D. Supplements are available from BioControl.

The final volume of sample/medium mixture in the container should be 10 ± 0.2 mL. Mix well.

**c.** Remove the lid from the Simplate® device and pour the sample/ medium mixture onto the center of the plate (Figure 1). Immediately replace the lid.

**b.** Remove the lid from the Simplate® device. If prepared sample size is 1.0 mL, pipette it onto the center of the device (Figure 2). Overlay the sample with 9.0 mL of medium. **DO NOT** count this media addition as a dilution.

**c.** For 0.1 mL of prepared sample, overlay it with 9.9 mL of medium. This is an additional 10 – fold dilution.

The final volume of sample/medium mixture on the plate should be 10 ± 0.2 mL.

Immediately replace the lid.

**d.** Gently swirl to distribute the sample/medium mixture into all the wells (Figure 3). The plate may be held with both hands and tilted slightly to help distribute the liquid into the wells.

**e.** Pour off excess medium by holding the lid against the plate on either side of the sponge cavity. Tip the plate toward you to allow liquid to drain into the sponge (Figure 4). DO NOT be concerned if partially filled wells are present. Wells containing partial volume of liquid will turn positive in the presence of viable fungi. Observe the background color of the wells. Background is defined as the color of the sample/medium mixture inside the wells.

**f.** DO NOT invert the Simplate® device. Incubate at room temperature (22 – 25 °C) for 56 – 72 h in the dark. If sample being analyzed is known to contain slow-growing fungal populations, such as certain mold genera, incubation time may be extended to 72 h. For samples containing Supplement V, incubate the Simplate® devices for 72 h in the dark.

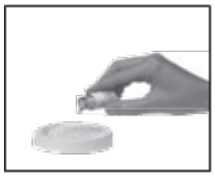

**Figure 1**

For single test, pour sample/ medium mixture onto the center of the plate.

**Figure 2**

For multiple tests, pipet sample onto center of the plate. Add rehydrated medium to make a final volume of 10 ± 0.2 mL.

**Figure 3**

Cover plate, gently swirl to distribute the sample into all of the wells.

**Figure 4**

Holding the cover, tip the plate toward you to allow liquid to drain.

## C. Reading and Interpretation of Results

**a.** If reading is taken between 56 and 63 h after inoculation, place the Simplate® device on a flat 8 watt light box to help visualize positive results. After 63 h of incubation the use of light box is not necessary.

**b.** After incubation, observe color change of the liquid in the wells. Disregard particulate matter if present. Count the number of wells showing a color change from the background color. The most common color changes produced by fungi are red, white, peach, and orange.

**NOTE:** For samples containing Supplement V, count only the number of wells that fluoresce blue by holding a UV light (366 nm wavelength) approximately 5 cm (2 inches) above the Simplate® device. Do not count non-fluorescent wells that only exhibit a color change.

**c.** To determine the population, perform the following calculations:

1. Count the number of positive wells on the plate.
2. Use the Simplate® Conversion Table to determine the total number of fungi per plate.

**d.** To calculate the number of **fungi per g (mL),** multiply the count **C(c)** (2) by the appropriate dilution factor (see sections **A** and **B**).

### D. Product and Storage Information

**a.** Store dehydrated medium away from direct light between 2-30 °C.

**b.** **DO NOT** use expired medium.

**c.** Store containers of reconstituted medium in the dark between 15 and 25 °C and use within 12 h.

**d.** Handle and dispose of incubated medium in a biological waste container and sterilize according to Good Laboratory Practices.

## Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.
BioControl Systems, Inc is an affiliate of``` Merck KGaA, Darmstadt, Germany.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

sigmaaldrich.com



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