

# Reading the Results

## Evaluation

Colonies of yeast species appear pink due to the assimilation of Rose Bengal. To determine colony counts on membrane filters, use a low-power binocular dissecting scope with a cool-white light source. Count all colonies on the membrane where there are 2 or fewer colonies per square. For 3-10 colonies per square, count 10 squares and obtain an average count per square. For 10-20 colonies per square, count 5 squares and obtain an average count per square. Multiply the average count per square by 100 and divide by the sample volume to give colonies per milliliter. If there are more than 20 colonies per square; record the count as >2000 per sample volume.

## Limitations

Although this medium is selective primarily for fungi, microscopic evaluation is recommended for presumptive identification. Due to the selective properties of the medium and specimen type being cultured, some strains of fungi may be inhibited or fail to grow entirely. Care should be taken not to expose this medium to light, since photodegradation of Rose Bengal yields compounds that are toxic to fungi.

InTray Rose Bengal w/ Chloramphenicol is an agar medium that is susceptible to condensation collection within the inner seal, especially when stored at low temperatures and/or having been exposed to extreme temperature fluctuations. If moisture is visible on the surface of the InTrays, dry them (with the seal removed and InTray label in a position allowing for air flow) under a BSL-2 cabinet just prior to inoculation. There should be no visible droplets of moisture on the surface of the agar when they are inoculated. The surface of the dried medium should be smooth and should not show signs (webbed ribbing pattern on the agar surface) of desiccation.

## References

1. American Health and Public Association. 1948. Standard Methods for the examination of dairy products, 9th ed. American Health and Public Association, New York, N.Y.
2. U.S. Environmental Protection Agency, 1997. EPA method 1600: Membrane filter test method for enterococci in water. USEPA EPA-821-R-97-004 Washington, D.C

Symbol glossary: [biomeddiagnostics.com/l/symbol-glossary](https://www.biomeddiagnostics.com/l/symbol-glossary)

## Document Revision History

### Rev. B, June 2020

New format; added new catalog numbers, limitation about condensation, document revision history, reference to online symbol glossary; specified 18–25°C instead of room temperature; removed organism from Quality Control section that are not tested for in-house.



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# InTray<sup>®</sup> Rose Bengal Agar w/ Chloramphenicol

REF 11-413-001	$\Sigma$ 5
REF 11-413-002	$\Sigma$ 20

A selective culture system for isolation and enumeration of yeast and molds.

For *In Vitro*, Analytical Use Only.  
Not for use in diagnostic procedures



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Certificate  
of Analysis

# Introduction

## Intended Use

InTray® Rose Bengal Agar test with antimicrobial supplement Chloramphenicol utilizes a highly selective, differential medium for use in the isolation and enumeration of yeasts and molds from soil, sewage and foods.<sup>1</sup>

## Description and Principle

This device is designed to interface directly with standard methods for bacterial testing of water samples using the Membrane Filter (MF) Technique as stated by the U.S. Environmental Protection Agency (USEPA). Rose Bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of more rapidly growing molds. This restriction aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. The Rose Bengal is taken up by the yeast and mold colonies, facilitating their recognition and enumeration. Chloramphenicol is employed in this medium as a selective supplement, because of its heat stability and broad antibacterial spectrum.

## Reagents and Appearance

This product contains Soy Peptone, Dextrose, Monopotassium Phosphate, Magnesium Sulfate, Rose Bengal, Chloramphenicol and agar, with a final pH of 7.2 ± 0.2 at 25°C

## Precautions, Safety and Disposal

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Do not use if package is damaged.

Once the tray has been inoculated and resealed, re-open only in a biological safety cabinet. Because of the potential for containing infectious materials, prior to disposal, sterilize the tray by autoclaving at 121°C for 20 minutes.

## Storage

Upon receipt, store InTray Rose Bengal w/ Chloramphenicol at 2 to 8°C. Avoid freezing or prolonged storage at temperatures greater than 40°C. Do not use if the medium shows signs of deterioration or contamination.

## Shelf Life

InTray Rose Bengal w/ Chloramphenicol expires 12 months from the date of manufacture.

# Procedure

## MF technique:

Select the sample volume to be examined in accordance with the information given in the membrane filtration technique. Using sterile forceps, place a sterile membrane filter on the filtration apparatus. Filter the sample through the membrane (100mL sample is passed through a 47 mm membrane filter) and rinse with an appropriate amount of sterile water.<sup>2</sup>

## Materials Provided

- InTray Rose Bengal w/ Chloramphenicol

## Materials Required but Not Provided

- Sterile inoculation tool (forceps)
- Sterile 47 mm membrane filter (for MF only)
- Laboratory incubator capable of incubation at 18-30°C in the dark

## Prepare InTray

Allow the InTray to warm to 18-25° C before inoculation. Manually pull the lower right corner back, completely exposing the protective seal. Remove the seal by pulling the tab and discard.

**Do not remove or alter the white filter strip over the vent hole!**

## Inoculate Sample

Using sterile technique, remove the filter from the MF apparatus and gently apply it (grid side up) to the surface of the agar in the InTray. Firmly reseal InTray by pressing the edges of the label and the plastic tray together all around the perimeter.

## Reseal InTray

Complete the label with sample information per your laboratory requirements.

## Incubation

Incubate inoculated trays for up to 7 days at 18-30°C, in the dark. The specificity of the test is dependent on the incubation temperature. Please consult relevant literature on the appropriate temperature for the microbe of interest before use

## Quality Control

This product has been tested and meets the CLSI (formerly NCCLS) Approved Standard for commercially prepared media (M22-A3). At the time of manufacture, quality control testing is performed on each lot of InTray Rose Bengal with Chloramphenicol. The ability of the media to support growth and demonstrate expected morphology is verified by lot. Refer to the COA for lot-specific information.

## Recommended strains for QC testing of InTray Rose Bengal w/ Chloramphenicol

Test Strain	ATCC®	Expected Result
<i>A. brasiliensis</i>	16404	Good
<i>C. albicans</i>	60193	Significant Inhibition
<i>E. coli</i>	25922	Significant Inhibition