After 24-48 hours incubation at 35-37°C, the stock cultures are kept in the refrigerator. Transfers are made at monthly intervals in triplicate.

### Inoculum

Inoculum for assay is prepared by subculturing a stock culture of *L. rhamnosus* ATCC 7469 into 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. Following incubation for 16-24 hours at 35-37°C, the culture is centrifuged under aseptic conditions and the supernatant liquid decanted. After washing 3 times with 10 mL sterile 0.85% saline, the cells are resuspended in 10 mL sterile 0.85% saline. The cell suspension is then diluted with sterile 0.85% saline, to a turbidity of 35-40% transmittance when read on the spectrophotometer at 660 nm. One drop of this latter suspension is then used to inoculate each of the assay tubes.

Riboflavin Assay Medium may be used for both turbidimetric and titrimetric determinations. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C, whereas titrimetric determinations are best made after 72 hours incubation at 35-37°C. Using Riboflavin Assay Medium, the most effective assay range is between 0.025 and 0.15 µg riboflavin.

### Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation, which influence the standard curve readings, cannot be duplicated exactly from assay to assay. The standard curve is obtained by using Riboflavin USP Reference Standard or equivalent at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 µg riboflavin per assay tube (10 mL).

The concentration of riboflavin required for the preparation of the standard curve may be prepared by dissolving 0.1 g of Riboflavin USP Reference Standard or equivalent in 1,000 mL of purified water by heating, giving a stock solution of 100 µg per mL. Dilute the stock solution by adding 1 mL to 999 mL purified water. Use 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 mL of the diluted stock solution per tube. Prepare the stock solution fresh daily.

## Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary by more than ±10%.

## Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.
5. Maintain pH below 7.0 to prevent loss of riboflavin.

## References

1. Snell and Strong. 1939. Ind. Eng. Chem. *11*:346.
2. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. II. AOAC International, Gaithersburg, Md.

## Availability

**Difco™ Riboflavin Assay Medium**

AOAC

Cat. No. 232510 Dehydrated – 100 g*

**Store at 2-8°C.*

# Rice Extract Agar

## Intended Use

Rice Extract Agar is used for promotion of chlamydospore formation by *Candida albicans* and *C. stellatoidea* as a means of differentiating them from other *Candida* species.

## Summary and Explanation

Rice Extract Agar was developed by Taschdjian to aid in the identification of chlamydospore-producing species of *Candida* so as to differentiate these species from others within the *Candida* genus.[1] Later, Taschdjian recommended inclusion of polysorbate 80 and the use of a lower concentration of medium (13 g/L) to enhance the formation of chlamydospores.[2]

Rice Extract Agar with 2% dextrose may be used to promote chromogenesis and, therefore, is helpful in distinguishing *Trichophyton rubrum* from *T. mentagrophytes*.

## Principles of the Procedure

The rice extract provides the nutrients required for the growth of *Candida* species. The addition of polysorbate 80 stimulates chlamydospore formation due to its content of oleic acids. Chlamydospore production is also favored by the use of a lower concentration, 13 g/L, although the medium can be prepared at a higher concentration (25 g/L).

The addition of 2% dextrose enhances chromogenesis in *T. rubrum*.

## Formula

**BBL™ Rice Extract Agar**

| Approximate Formula* Per Liter | | |
|---|---|---|
| White Rice, Extract from (solids) | 5.0 | g |
| Agar | 20.0 | g |

**Adjusted and/or supplemented as required to meet performance criteria.*

## Directions for Preparation from Dehydrated Product

1. Suspend 25 g of the powder in 1 L purified water. To promote chlamydospore formation, suspend 13 g of the powder in 1 L of purified water.
2. Add 10 mL polysorbate 80. Mix until a uniform suspension is obtained.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Dispense and autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

## User Quality Control

### *Identity Specifications*

**BBL™ Rice Extract Agar**

| | |
|---|---|
| Dehydrated Appearance: | Fine, homogeneous, free of extraneous material. |
| Solution: | 1.3% solution, soluble in purified water upon boiling. Solution is pale, yellow to tan, moderately hazy to hazy. |
| Prepared Appearance: | Pale, yellow to tan, moderately hazy to hazy. |
| Reaction of 1.3% Solution at 25°C: | pH 6.6 ± 0.2 |

### *Cultural Response*

**BBL™ Rice Extract Agar**

Prepare the medium per label directions and test for chlamydospore production. Using fresh cultures, streak two parallel lines approximately 1.5 cm long and 1.0 cm apart. Make an S-shaped streak lightly back and forth across the two parallel streak lines. Place a coverslip over the streak marks. Incubate at 20-25°C for 3-5 days and examine microscopically.

| ORGANISM | ATCC™ | RECOVERY | CHLAMYDOSPORES |
|---|---|---|---|
| *Candida albicans* | 10231 | Good | + |
| *Candida albicans* | 60193 | Good | – |

## Procedure

1. For use in the detection of chlamydospore formation. Inoculate the culture by cutting slits into the agar with an inoculating needle. Cover the inoculated slits with sterile coverslips. Seal the plates to avoid moisture loss and incubate at room temperature for 24-48 hours and up to 14 days before discarding as negative.
2. For use in the promotion of chromogenesis in *T. rubrum*. Streak-inoculate tubed medium slants. Tighten caps after inoculation and then loosen slightly. After incubation for 2-3 days, caps should be retightened to prevent further evaporation of water. Incubate tubes at room temperature for up to 14 days.

## Expected Results

After 24-48 hours, most strains of *C. albicans* and *C. stellatoidea* will have formed typical chlamydospores.[3] Invert the plate and examine microscopically (100× magnification) for chlamydospore formation along the line of inoculation.

Growth of *T. rubrum* is pink to red on medium containing dextrose and, therefore, it is distinguishable from *T. mentagrophytes*.

## Limitation of the Procedure

Polysorbate 80 enhances chlamydospore production by *C. albicans* and *C. stellatoidea*; however, it also enhances chlamydospore formation in other *Candida* species. Therefore, it is necessary to use additional media for species identification.[4]

## References


1. Taschdjian. 1953. Mycologia *45*:474.
2. Taschdjian. 1957. Mycologia *49*:332.
3. Cooper and Silva-Hutner. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.


## Availability

**BBL™ Rice Extract Agar**

Cat. No. 211567 Dehydrated – 100 g

*Europe*

Cat. No. 254420 Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*

# Rogosa SL Agar • Rogosa SL Broth

## Intended Use

Rogosa SL Agar and Rogosa SL Broth are used for cultivating oral, vaginal and fecal lactobacilli.

## Summary and Explanation

Rogosa SL Agar and Broth, also known as RMW Agar/Broth, are a modification of media described by Rogosa, Mitchell and Wiseman.[1,2] These media are used for isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs.[3,4] The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

## Principles of the Procedure

Peptone provides carbon and nitrogen. Yeast extract is a source of trace elements, vitamins and amino acids. Dextrose, arabinose and saccharose are carbohydrate sources that provide carbon. Sodium acetate and ammonium citrate inhibit streptococci, molds and other oral microbial flora and restrict swarming. Monopotassium phosphate provides