Procedure

Consult appropriate references for information about the processing and inoculation of specimens.[2]

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. The tubed slants also should be incubated at 25-30°C. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Expected Results

Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by the antibiotics in Inhibitory Mold Agar and Inhibitory Mold Agar with Gentamicin.[3]

References

1. Ulrich. 1956. Abstr. M75, p. 87. Bacteriol. Proc. Soc. Am. Bacteriologists. 1956.
2. Miller and Holmes. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Ajello, Georg, Kaplan and Kaufmann. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Inhibitory Mold Agar

BS10 CMPH MCM7

Cat. No.	292846	Dehydrated – 500 g
	212254	Dehydrated – 5 lb (2.3 kg)
	297799	Prepared Plates (Deep Fill) – Pkg. of 10*
	298191	Prepared Plates (Deep Fill) – Ctn. of 100*
	297276	Prepared Slants – Pkg. of 10*
	297826	Transgrow-style Bottle – Pkg. of 10*
	297757	Transgrow-style Bottle – Ctn. of 100*

BBL™ Inhibitory Mold Agar with Gentamicin

BS10 CMPH MCM7

Cat. No.	297800	Prepared Plates (Deep Fill) – Pkg. of 10*

**Store at 2-8°C.*

Inositol Assay Medium

Intended Use

Inositol Assay Medium is used for determining inositol concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Inositol Assay Medium, a modification of the formula described by Atkin et al.,[1] is used in the microbiological assay of inositol using *Saccharomyces cerevisiae* ATCC™ 9080 (*Saccharomyces uvarum*) as the test organism.

Principles of the Procedure

Inositol Assay Medium is an inositol-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *S. cerevisiae* ATCC 9080. The addition of inositol in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

User Quality Control

Identity Specifications

Difco™ Inositol Assay Medium

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	6.1% (single strength) solution, soluble in purified water upon boiling. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 6.1% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Difco™ Inositol Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Saccharomyces cerevisiae* ATCC™ 9080 when prepared in single strength and supplemented with inositol. The medium should produce a standard curve using an inositol reference standard at 0.0 to 10.0 µg per 10 mL. Incubate tubes with caps loosened at 25-30°C for 20-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Formula

Difco™ Inositol Assay Medium

Approximate Formula* Per Liter		
Dextrose	100.0	g
Potassium Citrate	10.0	g
Citric Acid	2.0	g
Monopotassium Phosphate	1.1	g
Potassium Chloride	0.85	g
Magnesium Sulfate	0.25	g
Calcium Chloride	0.25	g
Manganese Sulfate	50.0	mg
Ferric Chloride	50.0	mg
DL-Tryptophan	80.0	mg
L-Cystine	0.1	g
L-Isoleucine	0.5	g
L-Leucine	0.5	g
L-Lysine	0.5	g
L-Methionine	0.2	g
DL-Phenylalanine	0.2	g
L-Tyrosine	0.2	g
L-Asparagine	0.8	g
DL-Aspartic Acid	0.2	g
DL-Serine	0.1	g
Glycine	0.2	g
DL-Threonine	0.4	g
L-Valine	0.5	g
L-Histidine	124.0	mg
L-Proline	0.2	g
DL-Alanine	0.4	g
L-Glutamic Acid	0.6	g
L-Arginine	0.48	g
Thiamine Hydrochloride	500.0	µg
Biotin	16.0	µg
Calcium Pantothenate	5.0	mg
Pyridoxine Hydrochloride	1.0	mg

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

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 12.2 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL.
6. Autoclave at 121°C for 5 minutes.

Procedure

Remove a loopful of culture from a stock culture slant of *S. cerevisiae* ATCC 9080 and suspend it in 10 mL sterile 0.85% saline. Centrifuge cells at moderate speed for 10 minutes. Decant the supernatant and resuspend cells in 10 mL 0.85% sterile saline. Wash the cells three times with 10 mL sterile 0.85% saline. After the third wash, resuspend the cells in 10 mL 0.85% saline. Dilute 1 mL of the cell suspension in 1000 mL of sterile 0.85% saline. This diluted suspension is the inoculum. Use 1 drop of inoculum suspension to inoculate each assay flask.

The concentrations of inositol required for the preparation of the standard curve may be prepared by dissolving 200 mg inositol in 100 mL purified water. Mix thoroughly. Dilute 1 mL of this solution with 999 mL purified water to make a final solution containing 2 µg inositol per mL. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per flask. Prepare this stock solution fresh daily.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can impact the standard curve readings and cannot always be duplicated. The standard curve is obtained by using inositol at levels of 0.0, 1, 2, 4, 6, 8 and 10 µg per assay flask (10 mL).

Following inoculation, flasks are incubated at 25-30°C for 20-24 hours. Place flasks in the refrigerator for 15-30 minutes to stop growth. Growth is measured turbidimetrically using any suitable spectrophotometer.

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. Use the results only if two-thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown 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.

Reference

1. Atkin, Schultz, Williams and Frey. 1943. End. & Eng. Chem., Ann. Ed. *15*:141.

Availability

Difco™ Inositol Assay Medium

Cat. No. 212222 Dehydrated – 100 g*

**Store at 2-8°C.*