

Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. 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.

Inoculation of Potato Dextrose Broth with pure cultures of yeasts can assist in their identification.

Expected Results

After sufficient incubation, the plates, which were streak-inoculated, should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. The colonies in pour plates should be counted and the results expressed as yeast and molds counts per gram or milliliter of material, taking into account the applicable dilution factor.

Growth from tubes inoculated with pure cultures may be used for biochemical and/or serological testing.

For broth, observe cultures for surface growth and pellicle formation.

Limitations of the Procedure

1. Heating Potato Dextrose Agar after acidifying hydrolyzes the agar and may destroy the solidifying properties.
2. Potato Dextrose Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Marshall, (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Potato Dextrose Agar

	AOAC	BAM	BS10	CCAM	CMPH	COMPF	MCM7	SMD	USP
Cat. No. 213300									
213400									
213200									

BBL™ Potato Dextrose Agar

	AOAC	BAM	BS10	CCAM	CMPH	COMPF	MCM7	SMD	USP
<i>United States and Canada</i>									
Cat. No. 296272									
297945									
221002									
297241									
299906									

Japan

Cat. No. 251545									
251821									
251544									

Difco™ Potato Dextrose Broth

Cat. No. 254920									
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*Store at 2-8°C.

Potato Flakes Agar • Potato Flakes CC Agar Potato Flakes Agar with Chloramphenicol and Gentamicin

Intended Use

These media are used in qualitative procedures for the cultivation of pathogenic and opportunistic fungi encountered in clinical mycology.

Summary and Explanation

Potato Flakes Agar induces sporulation, enhancing the production of morphological structures required for the identification of many pathogenic and opportunistic fungi.¹ The addition of chloramphenicol and cycloheximide (CC) or gentamicin provides selectivity for more effective isolation and identification of medically significant fungi.

Principles of the Procedure

The medium stimulates the production of morphological features, such as conidia configurations, improving the ability to identify fungi by their particular morphological structures. Correct identification of fungi causing human disease depends upon visualization of characteristic morphological features.

The antimicrobial agents chloramphenicol, cycloheximide and gentamicin are incorporated in various combinations to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.² Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide is an anti-fungal agent that inhibits saprophytic

fungi, while permitting the growth of pathogenic species. Gentamicin is an aminoglycoside antibiotic that inhibits growth of gram-negative bacteria.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.³⁻⁵

For isolation of fungi causing cutaneous mycoses, a nonselective medium should be inoculated along with a selective medium. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. 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 the media for growth. Microscopic examination of the colony aids in identification.⁶

References

- Rinaldi. 1982. *J. Clin. Microbiol.* 15:1159.
- Merz and Roberts. 1995. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
- Baron, Peterson and Finegold. 1994. *Bailey & Scott's diagnostic microbiology*, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.
- Kwon-Chung and Bennett. 1992. *Medical mycology*. Lea & Febiger, Philadelphia, Pa.
- Koneman, Allen, Janda, Schreckenberger and Winn. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven Publishers, Philadelphia, Pa.
- Larone. 1995. *Medically important fungi: a guide to identification*, 3rd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Potato Flakes Agar

B510 **CMPH** **MCM7**

Cat. No. 298328 Prepared Plates – Pkg. of 10*

BBL™ Potato Flakes CC Agar

Cat. No. 298327 Prepared Plates – Pkg. of 10*

BBL™ Potato Flakes Agar with Chloramphenicol and Gentamicin

Cat. No. 292259 Prepared Slants – Ctn. of 100*

*Store at 2-8°C.

Potato Infusion Agar

Intended Use

Potato Infusion Agar is used for cultivating *Brucella*, especially in mass cultivation procedures.

User Quality Control

Identity Specifications

Difco™ Potato Infusion Agar

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in purified water with 2% glycerol upon boiling. Solution is medium amber, slightly opalescent, with a slight precipitate.
Prepared Appearance:	Medium amber, slightly opalescent to opalescent with a slight precipitate.
Reaction of 4.9% Solution with 2% Glycerol at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Potato Infusion Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO₂ for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Brucella abortus</i>	4315*	10 ² -10 ³	Good
<i>Brucella melitensis</i>	4309*	10 ² -10 ³	Good
<i>Brucella suis</i>	4314*	10 ² -10 ³	Good
<i>Streptococcus pneumoniae</i>	6305	30-300	Good

*Minimally, one strain of *Brucella* should be used for performance testing. These ATCC strains should be used if available.

Summary and Explanation

Potato Infusion Agar is prepared according to the formula used by Stockman and MacFadyean for the isolation of *Brucella abortus*. Brucellosis is a zoonotic disease with a domestic-animal reservoir.¹ Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.¹

Brucella spp. grow on most standard laboratory media, including blood agar and chocolate agar, when incubated at 35°C in a CO₂-supplemented atmosphere; however, enriched media are preferred.² Potato Infusion Agar, enriched with glycerol, permits luxuriant growth of characteristic colonies of *B. abortus* from infected materials, and may be used with excellent results in mass cultivation of *Brucella* in the preparation of vaccines and antigens.

Principles of the Procedure

Infusion from potatoes, beef extract and peptone provide the nitrogen, vitamins and amino acids in Potato Infusion Agar. Dextrose and glycerol are used as a carbon source in this formula. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula

Difco™ Potato Infusion Agar

Approximate Formula* Per Liter

Potatoes, Infusion from 200 g	4.0	g
Beef Extract	5.0	g
Proteose Peptone	10.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

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