Formula

Difco™ Sabouraud Brain Heart Infusion Agar Base

Approximate Formula* Per Liter		
Brain Heart Digest	9.25	g
Proteose Peptone	5.0	g
Enzymatic Digest of Casein	5.0	g
Dextrose	21.0	g
Sodium Chloride	2.5	g
Disodium Phosphate	1.25	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 1 mL chloramphenicol solution (100 mg/mL) and, if desired, 10% sterile sheep blood.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens.

For isolation of fungi from potentially contaminated specimens, both a nonselective and a selective medium should be inoculated. 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

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine the 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 selective formulations.[3,4]

References

1. Gorman. 1967. Am. J. Med. Technol. *33*: 151.
2. Merz and Roberts. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.

Availability

Difco™ Sabouraud Brain Heart Infusion Agar Base

Cat. No. 279720 Dehydrated – 500 g

BBL™ Sabouraud Brain Heart Infusion Agar

BS10 MCM7

Cat. No.	297802	Prepared Plates (Deep Fill) – Pkg. of 10*
	298192	Prepared Plates (Deep Fill) – Ctn. of 100*
	297691	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide

Cat. No.	297803	Prepared Plates (Deep Fill) – Pkg. of 10*
	297692	Prepared Slants – Ctn. of 100*

BBL™ Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Gentamicin

MCM7

Cat. No. 297252 Prepared Slants – Pkg. of 10*

BBL™ Sabouraud Brain Heart Infusion Sheep Blood Agar with Chloramphenicol

Cat. No. 296307 **Mycoflask**™ Bottles – Pkg. of 10*

**Store at 2-8°C.*

Sabouraud Media (Low pH) Sabouraud Dextrose Agar • Sabouraud Dextrose Agar with Antimicrobics • Sabouraud Dextrose Agar with Lecithin and Polysorbate 80 • Sabouraud Dextrose Broth • Sabouraud Maltose Agar Sabouraud Maltose Broth • Fluid Sabouraud Medium

Intended Use

Sabouraud Dextrose Agar conforms with specifications of *The United States Pharmacopeia (USP)*.

Sabouraud Dextrose Agar is used in qualitative procedures for cultivation of pathogenic and nonpathogenic fungi, particularly dermatophytes. The medium is rendered more selective for fungi by the addition of antimicrobics. Sabouraud Dextrose Broth and Sabouraud Maltose Agar and Broth are also used for culturing yeasts, molds and aciduric microorganisms.

Fluid Sabouraud Medium is used for cultivating yeasts, molds and aciduric microorganisms and for detecting yeasts and molds in normally sterile materials.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Sabouraud Dextrose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Sabouraud Dextrose Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Fluid Sabouraud Medium

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 3.0% Solution at 25°C:	pH 5.7 ± 0.2

Difco™ Sabouraud Maltose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Sabouraud Maltose Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water. Solution is light amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, clear to slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ Sabouraud Dextrose Agar or Sabouraud Dextrose Broth

Prepare the medium per label directions. For agar, inoculate and incubate at 30 ± 2°C for 18-48 hours, or up to 7 days for *Trichophyton*. For broth, inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Aspergillus niger	16404	10^2-3×10^2	Good	Good
Candida albicans	10231	10^2-3×10^2	Good	Good
Lactobacillus rhamnosus	9595	10^2-3×10^2	N/A	Good
Saccharomyces cerevisiae	9763	10^2-3×10^2	Good	Good
Trichophyton mentagrophytes	9533	Undiluted	Good	N/A

Difco™ Fluid Sabouraud Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	10^2	Good
Candida albicans	10231	10^2	Good
Saccharomyces cerevisiae	9763	10^2	Good

Difco™ Sabouraud Maltose Agar or Sabouraud Maltose Broth

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Aspergillus niger	16404	10^2-3×10^2	Good	Good
Candida albicans	10231	10^2-3×10^2	Good	Good
Escherichia coli	25922	10^2-3×10^2	N/A	Good
Lactobacillus rhamnosus	9595	10^2-3×10^2	N/A	Good
Saccharomyces cerevisiae	9763	10^2-3×10^2	Good	Good
Trichophyton mentagrophytes	9533	10^2-3×10^2	Good	N/A

Continued

Sterile Pack **RODAC**™ environmental sampling plates, containing Sabouraud Dextrose Agar with Lecithin and Polysorbate 80, are used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Sterile Pack plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas and are also recommended for use in air sampling equipment, such as the Surface Air System.

Sterile Pack **Finger Dab**™ Isolator plates are intended for sampling gloved hands.

Summary and Explanation

Sabouraud Dextrose Agar is a general-purpose medium devised by Sabouraud for the cultivation of dermatophytes.[1] The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and slightly inhibitory to contaminating bacteria in clinical specimens.[2-4] This medium is recommended in the *USP* for use in performing total combined mold and yeast counts (Microbial Limit Tests).[5]

The addition of antimicrobics is a modification designed to increase bacterial inhibition.

RODAC™ (Replicate Organism Detection and Counting) environmental sampling plates are specially constructed so that

Identity Specifications

BBL™ Sabouraud Dextrose Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain a large number of minute to small tan specks.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is pale to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to medium, yellow to tan, clear to slightly hazy.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

BBL™ Sabouraud Dextrose Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	RECOVERY
Aspergillus niger	16404	Good
Aureobasidium pullulans	9348	Good
Blastomyces dermatitidis	56218	Good
Candida albicans	60193	Good
Cryptococcus neoformans	32045	Good
Microsporum audouinii	9079	Good
Nocardia asteroides	19247	Good
Penicillium roquefortii	9295	Good
Trichophyton mentagrophytes	9533	Good

Candida albicans
ATCC™ 10231

an agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. These plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.[6-10] After touching the surface to be sampled with the medium, the environmental sampling dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium.[11] Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures.

Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for the dextrose. It is a selective medium due to the acid pH. Davidson et al. reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of infections caused by *Microsporum audouini*, *M. lanosum* and *Trichophyton gypseum*.[12] Davidson and Dowding also used this medium in isolating *T. gypseum* from a case of tinea barbae.[13]

Sabouraud Maltose Broth is a modification of Sabouraud Dextrose Broth in which maltose is substituted for dextrose. It is selective due to its acid pH and is used for the detection of fungi.

Fluid Sabouraud Medium is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. The acid reaction of the final medium is inhibitive to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Principles of the Procedure

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Media are also provided with maltose substituted for the dextrose. Peptones are sources of nitrogenous growth factors. The carbohydrate provides an energy source for the growth of microorganisms. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-negative and gram-positive bacteria, and cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit yeasts or dermatophytes.[14]

Lecithin neutralizes quaternary ammonium compounds, and polysorbate 80 neutralizes substituted phenolic disinfectants.[15-18]

For the Sterile Pack products, the entire double-bagged product is subjected to a sterilizing dose of gamma radiation, thus the contents inside the outer bag are sterile.[19] This allows the inner bag to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. A third sterile bag is included as a transport device. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants and not pre-existing microorganisms in the medium that may have been introduced during manufacture. The **RODAC** plates have a marked grid to facilitate counting organisms. The Sterile Pack **Finger Dab** Isolator plates are triple-bagged and are intended for sampling gloved hands.

Formulae

Difco™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Dextrose	40.0	g
Agar	15.0	g

BBL™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Dextrose	40.0	g
Agar	15.0	g

Difco™ Sabouraud Dextrose Broth

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Dextrose	20.0	g

Difco™ Fluid Sabouraud Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3	5.0	g
Dextrose	20.0	g

Difco™ Sabouraud Maltose Agar

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Maltose	40.0	g
Agar	15.0	g

Difco™ Sabouraud Maltose Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Sabouraud Dextrose Agar – 65 g;
   BBL™ Sabouraud Dextrose Agar – 65 g;
   Difco™ Sabouraud Dextrose Broth – 30 g;
   Difco™ Fluid Sabouraud Medium – 30 g;
   Difco™ Sabouraud Maltose Agar – 65 g;
   Difco™ Sabouraud Maltose Broth – 50 g.
   Mix thoroughly.
2. Heat the agar media with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating which could cause a softer medium.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the containers at 25-30°C with increased humidity. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Liquefy the medium in pour tubes by heating in boiling water. Cool to 45-50°C and pour into sterile Petri dishes. Allow to solidify for a minimum of 30 minutes.

Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes. With prepared plates and **Mycoflask™** bottles, streak the specimen as soon as possible after it is received in the laboratory, using a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.[3,4]

For the Sterile Pack media, sample selected surfaces by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally as this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

After sufficient incubation, the containers should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.

Examine containers for fungal colonies exhibiting typical color and morphology.[20] Biochemical tests and serological procedures should be performed to confirm findings.

In the **RODAC** procedure, colonies are counted (fewer than 200 colonies for accurate counts) and expressed as either the number of colonies per **RODAC** plate or the number of colonies per cm.[2,21,22] Criteria for cleanliness of equipment and environment (surfaces) can be developed by using a database derived from repeated routine sampling of specific sites.[23]

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examination of organism smears.

Limitation of the Procedure

Some fungi may be inhibited by the acidic pH of the medium and by the antimicrobics in the selective media.[2-4]

References

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5. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
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17. Erlandson and Lawrence. 1953. Science *118*: 274
18. Brummer. 1976. Appl. Environ. Microbiol. *32*: 80.
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20. Larone. 1995. Medically important fungi: a guide to identification, 3rd ed. American Society for Microbiology, Washington, D.C.
21. Vanderzant and Splittstoesser (ed.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
22. Marshall (ed.) 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
23. ICMSF. 1988. Microorganisms in foods 4. Intern. Comm. on Microbiology Spec. for Foods. Blackwall Scient. Publs., Palo Alto, Calif.

Availability

Difco™ Sabouraud Dextrose Agar

BAM BS10 CCAM CMPH COMPF EP USP

Cat. No.	210940	Dehydrated – 100 g
	210950	Dehydrated – 500 g
	211661	Dehydrated – 2 kg
	210930	Dehydrated – 10 kg

BBL™ Sabouraud Dextrose Agar

BAM BS10 CCAM CMPH COMPF EP USP

Cat. No.	211584	Dehydrated – 500 g
	211585	Dehydrated – 5 lb (2.3 kg)
	293309	Dehydrated – 25 lb (11.3 kg)

United States and Canada

Cat. No.	221180	Prepared Plates (Deep Fill) – Pkg. of 20*
	221278	Prepared Plates (Deep Fill) – Ctn. of 100*
	221235	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	297739	Prepared Plates (150 x 15 mm-style), Deep Fill – Pkg. of 24*
	221012	Prepared Slants (A Tubes) – Pkg. of 10*
	221013	Prepared Slants (A Tubes) – Ctn. of 100*
	297072	Prepared Slants (C Tubes) – Pkg. of 10*
	297479	Prepared Slants (C Tubes) – Ctn. of 100*
	297812	Prepared Pour Tubes, 20 mL – Pkg. of 10*
	296182	Prepared Pour Tubes, 20 mL – Ctn. of 100*
	221136	**Mycoflask**™ Bottles – Pkg. of 10*
	221137	**Mycoflask**™ Bottles – Ctn. of 100*
	297720	Transgrow-style Bottles – Ctn. of 100*
	295699	Bottles, 1 oz. – Ctn. of 100*

Europe

Cat. No.	254039	Prepared Plates – Pkg. of 20*
	254083	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251180	Prepared Plates – Pkg. of 20*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol

MCM7

Cat. No.	221851	Prepared Plates (Deep Fill) – Pkg. of 20*
	221825	Prepared Slants (C Tubes) – Ctn. of 100*
	221314	**Mycoflask**™ Bottles – Pkg. of 10*
	221315	**Mycoflask**™ Bottles – Ctn. of 100*
	299098	Bottle, 500 mL – Pkg. of 10

BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide

Cat. No.	297649	Prepared Slants – Pkg. of 10*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin

MCM7

Cat. No.	296359	Prepared Plates – Pkg. of 20*

BBL™ Sabouraud Dextrose Agar with Lecithin and Polysorbate 80

Cat. No.	221233	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	292653	Isolator Pack, **Finger Dab**™ Prepared Plates (100 × 15 mm-style) – Pkg. of 10*
	292654	Isolator Pack, **Finger Dab**™ Prepared Plates (150 × 15 mm-style) – Pkg. of 5*

Difco™ Sabouraud Dextrose Broth

BAM

Cat. No.	238220	Dehydrated – 100 g
	238230	Dehydrated – 500 g
	238210	Dehydrated – 2 kg

Difco™ Fluid Sabouraud Medium

Cat. No.	264210	Dehydrated – 500 g

Difco™ Sabouraud Maltose Agar

Cat. No.	211020	Dehydrated – 500 g

Difco™ Sabouraud Maltose Broth

Cat. No.	242910	Dehydrated – 500 g

**Store at 2-8°C.*

Sabouraud Agar, Modified • Sabouraud Dextrose Agar, Emmons • Sabouraud Dextrose Agar, Emmons, with Antimicrobics

Intended Use

Sabouraud Agar, Modified (Emmons) and Sabouraud Dextrose Agar, Emmons are used in qualitative procedures for cultivation of dermatophytes and other pathogenic and nonpathogenic fungi from clinical and nonclinical specimens.

Sabouraud Dextrose Agar, Emmons is rendered selective by the addition of antimicrobial agents.

Summary and Explanation

Sabouraud Dextrose Agar was devised by Sabouraud for the cultivation of dermatophytes.[1] The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and inhibitory to contaminating bacteria in clinical specimens.[2] The acidic pH, however, also may inhibit some fungal species.[2-4] Emmons modified the original formulation by adjusting the pH close to neutral to increase the recovery of fungi and by reducing the dextrose content from 40 to 20 g/L.[4] The two base formulations offered differ in peptone content and amount of agar. The addition of antimicrobics further increases the selectivity of the medium.[3,4]

Principles of the Procedure

Peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-negative and gram-positive bacteria,