Chapter 2
Sabouraud Agar and Other Fungal Growth Media

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2.1 Sabouraud Dextrose Agar

2.1.1 History

Sabouraud agar medium was developed by the French dermatologist Raymond J. A. Sabouraud (pronounced sah-bū-rō′) in the late 1800s to support the growth of fungi, particularly dermatophytes [1, 2]. Sabouraud's medical investigations focused on bacteria and fungi that cause skin lesions, and he developed many agars and techniques to culture pathogens such as dermatophytes and *Malassezia* species. The long incubation period (multiple weeks) of dermatophytes and the need to avoid bacterial contamination while culturing them, was one driving force behind the development of this medium. Additionally, Sabouraud sought to provide a medium that would yield reliable results for fungal identification *across* laboratories. He recommended that all mycologists detail their exact media formulations and sources of ingredients as well as the temperatures and times of specimen incubation, in order to standardize observations and reduce media-derived sources of differences in appearance [3].

Ironically, given Sabouraud's original desire to standardize the construction of fungal media, there are currently many sources of confusion and variation in both the names and ingredients associated with Sabouraud agar, also called Sabouraud's agar (abbreviated either SDA or SAB). Due to the old-fashioned use of the term "dextrose" to refer to D-glucose, the medium has been referred to as Sabouraud dextrose agar as well as Sabouraud glucose agar, the name most appropriate and consistent with standard chemical nomenclature [3]. Finally, a more recent modification of Sabouraud agar by Emmons is either called Sabouraud agar (Modified), or Sabouraud agar, Emmons [4]. Many of the historical details behind these names and ingredient variations are described in Odds' excellent review article [3].

2.1.2 Theory

Sabouraud agar is a selective medium that is formulated to allow the growth of fungi and inhibit the growth of bacteria. The available means of inhibiting bacterial growth in Sabouraud's pre-antibiotic era was via an acidic medium (pH 5.6). Currently, the addition of antibiotics or antimicrobials to the acidic medium is used to inhibit

bacterial growth (and sometimes saprophytic fungi, depending on the particular antimicrobial used).

Sabouraud agar medium is complex and undefined but contains few ingredients. Peptones, as soluble protein digests, are sources of nitrogenous growth factors that can vary significantly according to the particular protein source. The most variation is present in the source and method of these protein digests. Both Difco and BBL brand Sabouraud agars use pancreatic digests of casein as their peptone source, but they and other vendors also use a combination of pancreatic digest of casein and peptic digest of animal tissues. Sabouraud's original formulation contained a peptone termed "Granulée de Chassaing," which is no longer available. Mold morphology can vary slightly based on the peptones used, but pigmentation and sporulation can be consistent if one uses a consistent method of medium preparation, with the ingredients from the same source each time. Researchers should also explicitly describe the commercial or laboratory-prepared components used in their medium.

Sabouraud originally used sugar maltose as an energy source, and although this medium is still commercially available, glucose (formerly referred to as dextrose) is currently used most frequently. Glucose is present at a high level of 4% in Sabouraud's formulation to assist in vigorous fermentation and acid production by any bacteria present, inhibiting later bacterial growth [5].

In 1977, Emmons formulated an alternative version of Sabouraud's agar, which contains half the amount of glucose (2%) and a neutral pH of 6.8–7.0. The neutral pH seems to enhance the growth of some pathogenic fungi, such as dermatophytes. Agar concentrations ranging from 1.5–2.0% are found in commercial preparations of Sabouraud agar in both the original formula and Emmons modification and serve to solidify the medium in tube and plate medium.

2.1.3 Materials

Sabouraud agar can be either made from individual ingredients (see Tables 2.1 and 2.2), purchased either as a dehydrated powder that must be dissolved in water, autoclaved, and dispensed, or as a prepared medium that can be purchased in a tube, plate, or broth form from a variety of commercial sources. Various antimicrobials can be added to either the original recipe (Sabouraud agar) or Sabouraud agar, Modified/Emmons (see Table 2.2).

Table 2.1 Ingredients for Sabouraud agar and Sabouraud agar (Emmons)

Ingredient	Sabouraud agar (per liter)	Sabouraud agar (Emmons) (per liter)
Pancreatic digest of casein	10 g	10 g
Glucose	40 g	20 g
Agar	15–20 g	15–20 g

Table 2.2 Antimicrobial and other additives to Sabouraud agar

Ingredient	Amount (per liter)	Notes on preparation for use
Chloramphenicol*	50 mg	Dissolve in 10 mL 95% ethanol
Cycloheximide*	0.5 g	Dissolve in 2 mL acetone
Gentamicin sulfate	50 mg	Dissolve in 5 mL water; add before autoclaving
Lecithin	0.7 g	Add directly with other powdered medium ingredients before autoclaving
Tween 80	5 g	
Olive oil	N/A	Spread 0.1 mL sterile olive oil on surface of each agar plate

*Add these to molten, autoclaved media once it has been tempered in water bath to 45–50 °C

1. Deionized, distilled water.
2. Autoclave.
3. Graduated cylinder, 1000 mL.
4. Erlenmeyer flask (2 L if making 1 L of medium).
5. Analytical balance (if using antimicrobial agents).
6. Balance for weighing media ingredients.
7. Stir bar.
8. Stirring hotplate.
9. Slant tube rack for holding media tubes after autoclaving to solidify with a slanted surface.
10. Pancreatic digest of casein.
11. Glucose.
12. Chloramphenicol.
13. Gentamicin sulfate.
14. Cycloheximide.
15. Tween 80 (polysorbate 80).
16. Lecithin.
17. Olive oil, sterilized by autoclaving.
18. Sterile glass test tubes with caps.
19. Sterile Petri dishes, 100 mm diameter.

2.1.4 Method of Sabouraud Agar Preparation

2.1.4.1 Standard Preparation

1. Combine all ingredients, except any antimicrobials to be used, in ~900 mL of deionized water in a graduated cylinder while stirring with a magnetic stir bar.
2. Adjust to pH 5.6 with hydrochloric acid and adjust final volume to 1 L.
3. Transfer contents to a 2 L flask and boil on a heating/stirring plate while stirring, for 1 min.
4. Cover the opening of the flask loosely with aluminum foil and autoclave for 15 minutes at 121 °C under pressure of 15 lb. in^{-2}.

5. Cool to ~45–50 °C (roughly until one can support the flask underneath with an ungloved hand). If the antimicrobials chloramphenicol or cycloheximide are to be added, aseptically add them at this point and swirl medium gently (see Sect. 2.1.4.3).
6. Pour into Petri dishes or tubes and leave them at room temperature (15–30 °C) overnight to solidify and dry. When pouring plates, fill each Petri dish with at least 25 mL of medium to allow for medium dehydration during the longer incubation period required for fungi. If preparing tubes, slant the rack of covered tubes immediately after pouring in a slant tube rack, either at a 5° or 20° slant.
7. Store all media at 4 °C, regardless of whether they contain antimicrobials.

2.1.4.2 Method of Sabouraud Agar, Emmons Modification Preparation

1. Combine all ingredients, except any antimicrobials to be used, in ~900 mL of deionized water in a graduated cylinder while stirring with a magnetic stir bar.
2. Adjust to pH 6.8–7.0 with hydrochloric acid and adjust final volume to 1 L.
3. Transfer contents to a 2 L flask and boil on a heating/stirring plate while stirring, for one minute.
4. Cover the opening of the flask loosely with aluminum foil and autoclave for 15 minutes at 121 °C under pressure of 15 lb. in^{-2}.
5. Cool to ~45–50 °C (roughly until one can support the flask underneath with an ungloved hand). If the antimicrobials chloramphenicol or cycloheximide are to be added, aseptically add them at this point and swirl medium gently (see Sect. 2.1.4.3).
6. Pour into Petri dishes or tubes and leave at room temperature overnight to solidify and dry. Fill each Petri dish with at least 25 mL of medium to allow for medium dehydration during the longer incubation period required for fungi. If preparing tubes, slant the rack of covered tubes immediately after pouring in a slant tube rack, either at a 5° or 20° slant.
7. Store all media at 4 °C, regardless of whether they contain antimicrobials.

2.1.4.3 Variations on Standard Sabouraud Agar

Either Sabouraud agar or its Emmons version can be made more selective by adding antimicrobials (see Table 2.2). Antimicrobials commonly used are the aminoglycoside gentamicin, which inhibits gram-negative bacteria, chloramphenicol, which inhibits a wide range of gram-positive and gram-negative bacteria, and cycloheximide, which inhibits primarily saprophytic fungi, but not dermatophytes or yeasts [6, 7]. Chloramphenicol and gentamicin are used at 50 mg L^{-1} and cycloheximide at 0.5 g L^{-1} (See Table 2.2) [8]. Chloramphenicol and cycloheximide should only be added after the media has been autoclaved and then cooled to ~45–50 °C (See step 5 in Sect. 2.1.4.1). Gentamicin may be added to the medium ingredients before autoclaving.

Lecithin and Tween 80 are added to Sabouraud agar (Table 2.2) that is used in monitoring environmental surfaces that may have been treated with antiseptics and quaternary ammonium compounds, as these additives neutralize the cleaning compounds [9]. Sterile olive oil can be spread on the surface of Sabouraud agar plates to grow lipophilic *Malassezia* species [10].

2.1.5 Methods of Inoculation and Incubation

Sabouraud agar plates can be inoculated by streaking for isolation, as with standard bacteriological media, by exposing the medium to ambient air, or by tamping clinical sample material (e.g., hair, skin scrapings) onto the surface of the agar medium. When growing cultures in tubes, the caps should be screwed on loosely to admit air, as dermatophytes and most molds are obligate aerobes. Isolation of fungi is performed on plates, while slants are primarily used for maintaining pure, or stock, cultures once isolated. If using selective Sabouraud media, a control plate/tube without antimicrobials should also be inoculated for comparison. Typically, molds are incubated at room temperature or slightly warmer (25–30 °C), yeasts are incubated at 28–30 °C or both 30 °C and 37 °C if suspected to be dimorphic fungi.

Incubation times will vary, from approximately 2 days for the growth of yeast colonies such as *Malasezzia* to 2–4 weeks for growth of dermatophytes or dimorphic fungi such as *Histoplasma capsulatum*. Indeed, the incubation time required to acquire fungal growth is one diagnostic indicator used to identify or confirm fungal species. Dermatophytes, in particular, show characteristic incubation times ranging from 5–7 days (some *Epidermophyton* or *Microsporum* species) to 3–4 weeks for some *Trichophyton* species [11]. Cultures should be examined twice weekly and be held for 4–6 weeks before being reported as negative if infection by systemic agents such as *Histoplasma*, *Blastomyces*, or *Coccidioides* species is suspected.

2.1.6 Results

Depending on the antimicrobials used, different types of microorganisms and groups of fungi may grow on Sabouraud agar (See Table 2.3). Typically, saprophytic fungi are inhibited by cycloheximide and/or chloramphenicol, but yeasts and dermatophytes grow well in their presence. Conversely, even Sabouraud agar is unable to support the growth of a few dermatophytes in the absence of additives. For example, some *Trichophyton* species require additional growth factors, such as thiamine and inositol (*T. verrucosum*) or nicotinic acid (*T. equinum*), and may not grow well, if at all, on Sabouraud agar [12]. *T. mentagrophytes* and *T. rubrum,* however, grow well on Sabouraud agar. Similarly, the growth of *Malassezia* species is significantly impaired without the addition of olive oil overlaid on the surface of a Sabouraud agar plate [10].

Table 2.3 Expected growth of various microbes on Sabouraud agar containing antimicrobials

Microbe	Growth on SAB + CAM[a]	Growth on SAB + CHX[b]
Candida albicans	Yes	Yes
Cryptococcus neoformans	Yes	No
Aspergillus Niger	Yes	No
Trichophyton mentagrophytes	Yes	Yes
Microsporum audouinii	Yes	Yes
Blastomyces dermatitidis	Yes (mold phase at 25 °C)	Yes (mold phase at 25 °C)
	No (yeast phase at 37 °C)	No (yeast phase at 37 °C)
Histoplasma capsulatum	Yes (mold phase at 25 °C)	Yes (mold phase at 25 °C)
	No (yeast phase at 37 °C)	No (yeast phase at 37 °C)
Rhizopus spp.	Yes	No
Sporothrix schenckii	Yes	Yes
Penicillium roquefortii	Yes	No
Escherichia coli	No	No

[a]SAB + CAM = Sabouraud agar plus chloramphenicol
[b]SAB + CHX = Sabouraud agar plus cycloheximide

Mold morphology should be observed on both the top (obverse) and bottom (reverse) surfaces, as differences can be seen on each surface.

Variation from lot to lot as well as between commercial vendors of Sabouraud agar can significantly impact the qualitative and quantitative growth of fungi. One study comparing five different commercial preparations of Sabouraud glucose agar observed significant differences in the quantitation of yeasts as well as the color of *Aspergillus* colonies; however, the dermatophytes yielded reliably similar appearances on the five media sources tested [13].

2.2 Potato Dextrose Agar

2.2.1 History

The use of potato extract as a growth source in fungal media was published by New Zealand mycologist Ross Beever in 1970 [14], as part of a detailed study into the most efficacious component of potatoes as a growth medium. After analysis of the carbon, nitrogen, mineral salts, and other growth factor components of the potato extract medium, it was concluded that no one component was responsible for the stimulation of the growth of fungi.

2.2.2 Theory

Potato Dextrose Agar (PDA) contains dextrose as a carbohydrate source which serves as a growth stimulant and potato infusion that provides a nutrient base for luxuriant growth of most fungi. Agar is added as the solidifying agent. Potato Dextrose Agar (PDA) is a general-purpose medium for the identification, cultivation, and enumeration of fungi in foods and dairy products. Potato dextrose broth is a general-purpose broth medium for yeasts and molds.

Certain additives like tartaric acid, chloramphenicol, and chlortetracycline can be added as selective agents (Table 2.4). As recommended by the Food and Drug Administration, the American Public Health Association, and the Association of Analytical Chemists [15–18], PDA with tartaric acid is used for the plate count microbial examination of food and dairy products. The addition of chlortetracycline is recommended for the microbial enumeration of yeast and mold from cosmetics. PDA with chloramphenicol is recommended for the selective cultivation of fungi from mixed samples. PDA is also recommended by the U.S. Pharmacopeia for the preparation and maintenance of test strains used for microbial limit tests.

Potato infusion and dextrose promote luxuriant fungal growth, so PDA is also used for primary isolation of yeasts and molds from clinical specimens. Since it stimulates sporulation and pigmentation, it is also used for the differentiation of typical varieties of dermatophytes (species belonging to the genera *Epidermophyton*, *Microsporum* or *Trichophyton*) based on their pigment production and for the maintenance of their stock cultures [19].

2.2.3 Methods of Preparation

See Sect. 2.1.3 for general comments regarding needed materials.

Potato Dextrose Agar can be either made from individual ingredients, purchased as a dehydrated powder that must be dissolved in water, autoclaved, and dispensed, or as a prepared medium in a tube, plate, or broth format from a variety of commercial sources.

Table 2.4 Antimicrobial and other additives to Potato Dextrose Agar

Ingredient	Amount (per liter)	Purpose in medium
Tartaric acid	1.4 g	Lowers pH to 3.5; antibacterial for testing food products
Chlortetracycline[a]	40 mg	Antibacterial; for cosmetics testing
Sodium chloride	75 g	Inhibition of fast- growing molds
Chloramphenicol[a]	25 mg	Antibacterial
Copper sulfate	1 mg	Supports pigmentation

[a]See Table 2.2 for preparation information on adding antimicrobials to media

Table 2.5 Ingredients for Potato Dextrose Agar (commercial and manual)

Ingredient	Potato Dextrose Agar (commercial) (g/L)	Potato Dextrose Agar (manual) (g/L)
Potato extract*	4 g	–
Potato infusion	–	200 g
Dextrose	20 g	20 g
Agar	15 g	15 g
Final pH at 25 °C	5.6 ± 0.2	5.6 ± 0.2

*4 g of potato extract is equivalent to infusion from 200 g of potatoes

A survey of 10 companies' media found that five media sources contained insufficient copper, which reduced the pigmentation of the resulting colonies. Therefore, supplementation of the other ingredients of PDA with 1 μg mL^{-1} copper sulfate, or a trace minerals mixture, is recommended [20].

1. Suspend 39 g of dehydrated media (if commercially supplied) in 1 L of deionized water and mix thoroughly in a 2 L flask.
   Alternatively, to make medium from individual ingredients (See Table 2.5):
   - Boil 200 g of sliced potatoes in 500 mL distilled water until thoroughly cooked (about 1 h).
   - Filter infusion through cheesecloth/gauze, saving the filtrate, which is potato infusion.
   - In a 2 L flask, combine the potato infusion, dextrose (20 g), and agar (15 g), and bring the volume to 1 L.
2. Boil on a heating/stirring plate while stirring, for 1 min to dissolve the powder completely.
3. Cover the opening of the flask loosely with aluminum foil and autoclave 15 min at 121 °C under pressure of 15 lb. in^{-2}.
4. Cool to around 45–50 °C (roughly until one can support the flask underneath with an ungloved hand).
5. When modifications are needed in the standard media:
   (i) To decrease the pH of the agar medium to pH 3.5, add the specified amount of sterile tartaric acid. The amount of acid required for 1 L of sterile, cooled medium is approximately 10 mL of a 10% solution.
   Note: Do not reheat the medium after adjusting pH as it may hydrolyze the agar which can render the agar unable to solidify.
   (ii) If the antimicrobials, such as chloramphenicol or chlortetracycline or other additives like sodium chloride are to be added, aseptically add them at this point and swirl medium gently.

6. Pour into Petri dishes or tubes and leave at room temperature overnight to solidify and dry. When pouring plates, fill each Petri dish with at least 25 mL of medium to allow for medium dehydration during the longer incubation period required for fungi. If preparing tubes, slant the rack of covered tubes immediately after pouring in a slant tube rack, either at 5° or 20° slant.
7. Store prepared media away from direct light at 4 °C to 8 °C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions, this medium has a shelf life of 12 weeks.

2.2.4 Methods of Inoculation and Incubation

2.2.4.1 Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium: *Candida albicans* ATCC 14053 and *Aspergillus niger* ATCC 16404 will each produce growth on this medium.

2.2.4.2 Inoculation and Incubation

1. For yeast and mold counts in foods, a standard pour plate technique should be used, and the pH of the medium should be adjusted to approximately 3.5 with sterile tartaric aid.
2. For the cultivation and maintenance of pure cultures, tubed slants are used. They should be inoculated and incubated the same as a plated medium, below.
3. For other specimen processing, streak the specimen onto the medium with a sterile inoculating loop to obtain isolated colonies.
4. Plates can be incubated at various temperatures, depending on the application (molds typically use a lower temperature such as room temperature 20–25 °C while yeasts may require 25–30 °C) in an inverted position (agar side up) with increased humidity.
5. Cultures should be examined daily for fungal growth, with extended time periods up to at least one week for lower temperature incubations and several days for higher temperature incubations.

2.2.5 Results

After sufficient incubation culture plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Yeasts will grow as creamy to white colonies. Molds will grow as filamentous colonies of various colors. Further

microscopic examination and biochemical testing are required to identify the genus and species of the isolate.

The number of yeast or mold present in the particular test sample is determined by counting the colonies in pour plates and multiplying with the applicable dilution factor.

2.3 Bird Seed Agar

2.3.1 History

In 1962, Staib et al. described that incorporation of an extract of *Guizotia abyssinica* seed in a fungal medium resulted in the formation of brown colonies of *Cryptococcus neoformans* [21]. In 1966, Shields and Ajello modified Staib's *G. abyssinica* seed agar formulation by making the medium selective with the addition of the antimicrobial agent chloramphenicol [22]. In the literature, this medium is variously referred to as Staib medium, Bird seed agar, *Guizotia abyssinica* creatinine agar, niger seed creatinine agar, or thistle seed medium [23].

2.3.2 Theory

Bird Seed Agar is a selective and differential medium for the isolation of *Cryptococcus neoformans* from clinical specimens and differentiation of it from other microbes. The use of Bird Seed Agar as the primary culture medium for sputum and urine specimens from AIDS patients increases sensitivity for *C. neoformans* [24]. *Cryptococcus neoformans* produces dark colonies on this agar, unlike *Candida albicans,* which produces white colonies. *C. neoformans* is the only yeast known to produce this pigmentation [25].

The extract of *Guizotia abyssinica* seeds contains caffeic acid (3, 4-dihydroxycinnamic acid, an o-diphenol). Phenoloxidase enzyme produced by *C. neoformans* uses caffeic acid as a substrate and produces melanin. Melanin is absorbed by the yeast cell wall, yielding tan to reddish-brown pigmentation. It is also known as the phenoloxidase test.

Glucose is the energy source in the medium. *Cryptococcus* species use creatinine as their sole source of nitrogen [22]. Creatinine also enhances the melanization of some strains of *C. neoformans.* Agar is the solidifying agent. Chloramphenicol improves the recovery of *Cryptococus* species from specimens containing mixed flora by selecting against bacterial growth.

2.3.3 Methods of Preparation

See Sect. 2.1.3 for general comments regarding needed materials.

Bird Seed Agar can be prepared from individual ingredients (Table 2.6) by purchasing the seeds of *Guizotia abyssinica* (niger seed), grinding them, and adding filtrate with necessary ingredients, or by using a commercially available powder media that includes bird seed filtrate. Prepared plates or tubes are also commercially available.

The exact composition of the media supplied by different commercial suppliers varies; each mentions that they modified the original formula to suit performance parameters. Some of the modifications include a substitution of chloramphenicol for penicillin and streptomycin sulfate, preparing modified *G. abyssinicia* seed-based agar media by depleting or removing its constituents such as diphenyl, glucose, creatinine, etc. It was found that decreasing the sugar concentration resulted in the rapid development of brown pigmentation by *C. neoformans* [26].

2.3.3.1 Preparation of Bird Seed Agar

1. Suspend required quantity powder media (as per manufacturer's instruction), OR items a-c and f-g in Table 2.6, in a total of 1 l of distilled water in a 2 L flask.
2. Heat to boiling on a heating/stirring plate to dissolve the medium completely.
3. Cover opening of flask loosely with aluminum foil and autoclave 15 min at 121 °C under pressure of 15 lb. in^{-2}.
4. Cool to 45 °C before adding additives if needed (e.g., diphenyl can be added to inhibit saprotrophic fungi).
5. Mix well and pour into sterile Petri plates or tubes (see Sect. 2.1.4.1, step 6).
6. Store prepared medium at 2–8 °C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface.

Table 2.6 Ingredients for Bird Seed Agar

Ingredients	Shield's and Ajello's formulation (22)	Commercial preparation (7)
a) *Guizotia abyssinica* seeds	200 mL filtrate	50 g
b) Glucose/dextrose	10 g	1 g
c) Creatinine	780 mg	1 g
d) Chloramphenicol	50 mg	50 g
e) Diphenyl	100 mg	–
f) Monopotassium phosphate	–	1 g
g) Agar	20 g	15 g
Final volume	1 L	1 liter
Final pH at 25 °C	6.7 ± 0.2	6.7 ± 0.2

2.3.4 Method of Inoculation and Incubation

2.3.4.1 Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium: *Cryptococcus neoformans* ATCC 32045 will produce brown to black pigmented colony growth on this medium. *Escherichia coli* ATCC 25922 serves as a negative control, being partial to completely inhibited in its growth on Bird Seed Agar.

2.3.4.2 Inoculation and Incubation

1. Streak the specimen onto the medium with a sterile inoculating loop to obtain isolated colonies.
2. Incubate the plates at 25–30 °C in an inverted position (agar side up) with increased humidity.
3. Cultures should be examined at least daily for fungal growth and should be held for up to 4 weeks before being reported as negative.

After inoculation of the clinical specimen, and periodically examined for brown-colored, mucoid colonies, which later turn black or brown in the case of the genus *Cryptococcus*.

2.3.5 Results

Plates inoculated with suspected samples are observed after incubation at 25–30 °C for 2 weeks. The presence of golden brown to black pigmented smooth or mucoid colonies is indicative of *Cryptococcus neoformans*. Other species like *Cryptococcus laurentii, Saccharomyces cerevisiae*, etc., produce non-pigmented colonies, and *Candida* species appear as white colonies. However, pigmented colonies arising on Bird Seed Agar should be also grown on a non-differential medium such as Sabouraud dextrose agar to rule out a naturally pigmented strain or species [19].

2.4 Dermatophyte Test Medium

2.4.1 History

Dermatophyte Test Medium (DTM) was formulated by Taplin et al. in 1969 to rapidly diagnose dermatophytic (ringworm) infections in Vietnam War soldiers,

under conditions where experienced laboratory personnel and incubators were typically unavailable [27].

2.4.2 Theory

Dermatophyte Test Medium, or DTM, is essentially a selective and differential version of Sabouraud dextrose agar designed to indicate the presence of *Epidermophyton*, *Microsporum*, and *Trichophyton* spp. that cause dermatophytic infections. Amino acids, nitrogen- and carbon-containing compounds are provided by the soy peptone, and dextrose is the energy source for fungal growth. The acidic pH 5.6 favors fungal growth.

The original formulation included chlortetracycline HCl and gentamicin as antibacterial agents [27]. Current formulations include the antibiotics chloramphenicol (due to the relative unavailability of chlortetracycline) and sometimes gentamicin, which select against a wide range of bacteria. Cycloheximide selects against saprophytic fungi. The medium is made differential through the addition of the indicator dye phenol red. At acidic pH values below 6.8, it is yellow, and at alkaline pH values of 8.2 and above, is bright pink to red. Dermatophytes produce alkaline metabolites that turn the media bright pink or red color.

2.4.3 Method of Preparation

See Sect. 2.1.3 for general comments regarding needed materials.

DTM can be either made from individual ingredients (see Table 2.7), purchased as a dehydrated powder that must be dissolved in water, autoclaved, and dispensed (after addition of antimicrobials), or as a prepared medium that can be purchased in tube or plate form.

Table 2.7 Ingredients for Dermatophyte Test Medium

Ingredient	Amount (per liter)
Soy peptone	10.0 g
Dextrose	10.0 g
Cycloheximide[a]	0.5 g
Chloramphenicol[a]	0.05 g
Gentamicin sulfate[a]	0.1 g
Phenol red	0.2 g
Agar	20.0 g

[a]See Table 2.2 for preparation information on adding antimicrobials to media

Follow steps 1–5 listed in Sect. 2.1.4.1 Standard Preparation. After cooling the sterilized media, add the appropriate antimicrobials for DTM (Table 2.7) and pour them into plates or tubes.

There is a modification of DTM, called dermatophyte isolation medium DIM [28], which uses the pH indicator dye bromocresol purple in place of phenol red, as well as penicillin and streptomycin as its antibiotics. It also uses cycloheximide, at 4 g L^{-1}, which is eight times the amount found in DTM. However, at its recommended incubation temperature of 37 °C (but not a lower temperature of 30 °C), high false-negative rates for common *Trichophyton* species were noted, perhaps due to the high levels of cycloheximide [29]. Furthermore, the dimorphic fungus *Coccidioides immitis* grew as a white mold at 37 °C that resembled the hallmark dermatophyte appearance, thereby constituting a false-positive issue with this medium [29]. Consequently, this medium formulation, as published, is not the most useful for presumptive identification of dermatophyte infections.

2.4.4 Methods of Inoculation and Incubation

Suitable samples for DTM inoculation include hair, nails, and skin scrapings. Clean the skin or body surface with alcohol before using a new toothbrush or other small brush to obtain a surface sample. Bringing the medium to room temperature helps facilitate more rapid fungal growth. Follow the instructions listed in Sect. 2.1.5. Lay the sample firmly on top of, but not into, the medium.

Loosely capped, inoculated tubes or plates should be incubated at room temperature or around 25 °C, and should be evaluated for red medium color change daily, up to 10–14 days.

2.4.5 Results

Two observations constitute a true positive reaction on DTM: white or buff-colored mold growth (containing aerial hyphae), and red-colored medium. Both the fungal growth and the medium color change to pink/red should appear at the same time, which may be as short as three to 5 days, depending on the inoculum. A red-colored medium arising after approximately 10–14 days likely represents contaminating fungal growth [27]. Various false-negative appearances are possible, such as saprophytes that grow as dark-colored molds although they turn the medium red/pink. Alternately, bacterial or yeast will grow as white- or light-colored creamy colonies, and thus easily distinguished from the mold-like appearance of dermatophytes.

DTM is primarily a screening medium and is not suitable for identification beyond presumptively belonging to the three species of dermatophytes.

2.5 Safety Notes

Fungi often produce spores that are easily dispersed into the laboratory upon the opening of plates.

Plates should be incubated with the lid on the top (as opposed to the typical practice of inverting microbiological plates for incubation) to avoid spreading spores when the plates are opened. After growth, plates should be wrapped in Parafilm to maintain them securely closed for storage and transport. Plate or tube cultures should be opened only within a class II biological safety cabinet to avoid contamination of laboratory spaces with fungal spores, possible infection of individuals by pathogenic fungi, or induction of allergic responses. See Chap. 1 for detailed procedures and guidelines.

Because the growth of large numbers of fungi can pose a potential infection hazard, measures must also be taken to prevent infection of laboratory researchers. Note that some fungi are biosafety level one (BSL-1) while most are BSL-2 [30]. The American Society for Microbiology strongly recommends that environmental enrichment experiments should only be performed in BSL-2 laboratories [31]. The following precautions apply to the use of any fungal medium:

1. Soil, water, and other materials directly obtained from the environment that typically contain infectious organisms should be handled according to the biosafety level of that infectious agent.
2. Cultures of enriched microorganisms derived from environmental samples should be handled using BSL-2 precautions.
3. Mixed, enriched, or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a Class II biosafety cabinet if available.
4. Researchers should be aware of working in regions with endemic fungi capable of causing systemic infections and should avoid environmental isolations.

Some safe (BSL-1) fungi for student experimentation and handling include the molds *Penicillium camemberti* and *P. roqueforti* (used in making cheeses), *Rhizopus stolonifor* (used in making tempeh), *Aspergillus* species (except *A. fumigatus* and *A. flavus*), the yeasts *Saccharomyces cerevisiae, Rhodotorula rubrum,* and *Neurospora crassa*.

2.6 Additional Fungal Growth Media

Table 2.8 Other commonly used fungal isolation and growth media

Culture Media	Essential ingredients	Intended use
Inhibitory Mold agar (IMA)	Tryptone, beef extract, yeast extract, starch, dextrin, chloramphenicol, gentamicin, and saline buffer	Recovery of fungi from specimens that contain bacterial microbiota

(continued)

Table 2.8 (continued)

Culture Media	Essential ingredients	Intended use
Mycosel/ Mycobiotic agar	Papaic digest of soybean meal, dextrose, cycloheximide, chloramphenicol, agar	Highly selective medium; recommended for isolation of pathogenic fungi from materials containing a large amount of fungal and bacterial flora
Potato flake agar	Potato flakes, dextrose, agar	Primary recovery of saprophytic and dimorphic fungi, particular fastidious and slow-growing strains
Cornmeal agar/cornmeal tween agar	Cornmeal, tween 80, agar	Stimulation of chlamydospore formation in yeasts
Rice starch agar	Cream of rice, tween 80, agar	Production of chlamydospore in *Candida albicans*
Brain-heart infusion agar	Brain heart infusion, glucose, L-cysteine hydrochloride, agar	Growth of fastidious pathogenic fungi such as *Histoplasma capsulatum* and *Blastomyces dermatitidis*
Czapek-dox agar	$NaNO_3$, K_2HO_4, KCl, $MgSO_4$, $FeSO_4$, glucose, agar	Identification of *aspergillus* and P*enicillium* species
CHROMagar Candida medium	Peptone, glucose, chloramphenicol, 'chromogenic ix', agar	Selective and differential chromogenic medium for the isolation and identification of various *Candida* species

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