Recommended Growth Requirements for Microorganisms

Selection Of Growth Requirements

1. Primary growth on a nonselective agar medium is preferred. Primary growth in a fluid medium should only occur in special instances or when recommended. Because of the manipulations required during hydration, it is difficult to obtain purity of a lyophilized strain in a fluid medium. A contaminant may completely overgrow and obscure the presence of the lyophilized strain.

2. The following information lists which method should be used to grow the various microorganism species. Descriptions of methods follow the microorganism list.

Microorganism	Method	Notes
Acetobacter sp.	Method 3	Incubate at 25°C in CO_2 for 3 to 4 days.
Achromobacter sp.	Method 1	
Acinetobacter sp.	Method 1	
Actinobacillus sp.	Method 3	
Actinomyces sp.	Method 4	
Aerococcus sp.	Method 1	
Aeromonas sp.	Method 2	*A. hydrophila* should be incubated at 30°C. *A. salmoncida* should be incubated at 25°C.
Aggregatibacter sp.	Method 3	
Alcaligenes sp.	Method 1	
Alicyclobacillus sp.	Method 12	*A. acidoterrestris*, Microbiologics 0265, should be incubated at 45°C.
Alternaria sp.	Method 5	
Alloiococcus sp.	Method 2	
Amylomyces sp.	Method 5	
Aneurinibacillus sp.	Method 1	
Aquaspirillum sp.	Method 1	Incubate at 25°C for 6 days.
Arcanobacterium sp.	Method 2	
Arthrobacter sp.	Method 1	Incubate at 25°C.
Aspergillus sp.	Method 5	*A. flavus* does not grow well on Standard Methods Agar (Plate Count Agar).
Aureobasidum sp.	Method 5	

Microorganism	Method	Notes
Bacillus sp.	Method 1	Some *Bacillus* sp. demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2°C to 8°C.
Bacteroides sp.	Method 4	*B. ureolyticus* should be incubated 5 days. The colonies are very small. Several subculture plates may need to be inoculated in order to have sufficient quantity of the microorganism for testing.
Bifidobacterium sp.	Method 4	*B. animalis* subsp. *animalis* only grows well on Anaerobic Blood Agar or Tryptic Soy Agar.
Bordetella bronchiseptica	Method 15	
Bordetella parapertussis	Method 16	
Bordetella pertussis	Method 16	
Brevibacillus sp.	Method 1	
Brevundimonas sp.	Method 1	
Brochothrix sp.	Method 1	Incubate at 25°C.
Budvicia sp.	Method 1	Incubate at 25°C.
Burkholderia sp.	Method 1	
Chaetomium sp.	Method 5	
Campylobacter sp.	Method 6	Chocolate agar is the best medium for the primary growth of *C. jejuni*. Do not open the inoculated agar medium petri plate for the first 48 hours.
Candida sp.	Method 5	
Capnocytophaga sp.	Method 3	
Cedecea sp.	Method 1	
Cellulosimicrobium sp.	Method 1	
Chryseobacterium shigense	Method 1	Incubate at 30°C.
Citrobacter sp.	Method 1	
Cladosporium sp.	Method 5	
Clostridium sp.	Method 4	*C. difficile*, *C. sordellii*, and *C. tetani* will only grow on Anaerobic Blood Agar. *C. perfringens* may not grow well on Nutrient Agar.
Corynebacterium sp.	Method 1	Use Method 2 to grow *C. urealyticum*.
Cronobacter sp.	Method 1	
Curtobacterium sp.	Method 1	

Microorganism	Method	Notes
Cryptococcus sp.	Method 5	*Cryptococcus* must be incubated at 25°C to assure growth. *C. gattii* grows best on Malt Extract Agar or Sabouraud Dextrose Emmons Agar.
		Cryptococcus grows poorly on non-selective Sheep Blood Agar.
Deinococcus sp.	Method 1	
Delftia sp.	Method 1	
Desulfotomaculum sp.	Method 17	
Edwardsiella sp.	Method 1	
Eggerthella sp.	Method 4	
Eikenella sp.	Method 3	
Elizabethkingia sp.	Method 1	
Enterobacter sp.	Method 1	
Enterococcus sp.	Method 1	
Erysipelothrix sp.	Method 2	
Escherichia coli	Method 1	
Exiguobacterium sp.	Method 1	
Finegoldia sp.	Method 4	Incubate 72 to 96 hours in anaerobic atmosphere.
Fluoribacter sp.	Method 8	
Fusarium sp.	Method 5	
Fusobacterium sp.	Method 4	
Gardnerella sp.	Method 9	
Gemella sp.	Method 4	
Geobacillus sp.	Method 1	*G. stearothermophilus* strains must be incubated at 55°C. *G. stearothermophilus*, Microbiologics 0137, does not grow on Sheep Blood Agar.
Geotrichum sp.	Method 5	
Granulicatella adiacens	Method 19	
Haemophilus sp.	Method 3	
Hafnia sp.	Method 1	
Issatchenkia sp.	Method 5	
Kingella sp.	Method 2	Incubate in 5 to 10% CO_2.

Microorganism	Method	Notes
Klebsiella sp.	Method 1	
Kloeckera sp.	Method 5	
Kocuria sp.	Method 1	*K. rosea* should be incubated at 25°C.
Lactobacillus sp.	Method 11	
Lactococcus sp.	Method 2	
Leclercia sp.	Method 1	
Legionella sp.	Method 8	
Listeria sp.	Method 1	
Lysinibacillus sp.	Method 1	
Macrococcus sp.	Method 1	
Malassezia sp.	Method 14	
Mannheimia sp.	Method 1	
Methylobacterium sp.	Method 1	Incubate at 25°C for 5 days. Grows best on Standard Methods Agar (Plate Count Agar). Does not grow on Tryptic Soy Agar (Soybean Casein Digest Agar) or nonselective Sheep Blood Agar.
		M. extorquens obtains good growth on R2A Agar in 72 hours at 30°C.
Microbacterium sp.	Method 1	Incubate at 30°C.
Micrococcus sp.	Method 1	*M. luteus*, Microbiologics 0337 and 0689 perform best on Tryptic Soy Agar (Soybean Casein Digest Agar) or nonselective Sheep Blood Agar.
		M. luteus, Microbiologics 0689, should incubate on Standard Methods Agar (Plate Count Agar) for a minimum of 72 hours.
Microsporum sp.	Method 5	*M. canis* grows poorly on Sabouraud Dextrose Agar.
Moraxella sp.	Method 2	
Morganella sp.	Method 1	
Mucor racemosus	Method 5	
Mycobacterium sp.	Method 7	*M. gordonae*, *M. terrae* and *M. tuberculosis* may require up to one month incubation.
		M. haemophilium should be grown on Middlebrook 7H11 Agar and incubated at 30°C in 5 to 7% CO_2 for 3 to 4 weeks. An X factor strip must be place on the agar in order for the organism to grow.
Mycoplasma sp.	Method 18	

Microorganism	Method	Notes
Myroides sp.	Method 2	
Neisseria sp.	Method 3	Chocolate agar is the best medium for the initial growth of *Neisseria* species. Do not open the inoculated agar medium petri plate for the first 48 hours if using a candle jar.
Nocardia sp.	Method 1	
Novosphingobium sp.	Method 1	Incubate at 25°C.
Ochrobactrum sp.	Method 1	
Oligella sp.	Method 2	
Paecilomyces sp.	Method 5	
Paenibacillus sp.	Method 1	*P. larvae* should be incubated aerobically at 30°C.
Parabacteroides sp.	Method 4	
Parvimonas sp.	Method 4	*P. micra* requires 5 to 7 days of anaerobic incubation.
Pasteurella sp.	Method 2	
Pediococcus sp.	Method 11	*P. damnosus* may be grown in MRS broth at 25ºC for 48 to 72 hours. Subculture the broth to MRS Agar when it becomes cloudy. Incubate the agar at 25ºC in 5 to 7% CO_2 for 72 to 96 hours. Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25ºC in 5 to 7% CO_2 for 5 to 7 days.
Penicillium sp.	Method 5	
Peptoniphilus sp.	Method 4	Incubate 72 to 96 hours in anaerobic atmosphere.
Peptostreptococcus sp.	Method 4	
Plesiomonas sp.	Method 1	
Porphyromonas sp.	Method 4	5 to 7 days of anaerobic incubation is required.
Prevotella sp.	Method 4	5 to 7 days of anaerobic incubation is required.
Propionibacterium sp.	Method 4	3 to 5 days of anaerobic incubation is required.
Proteus sp.	Method 1	*P. hauseri* grows best on Blood and Tryptic Soy Agar.
Prototheca sp.	Method 5	
Providencia sp.	Method 1	
Pseudomonas sp.	Method 1	*P. fluorescens* and *P. protogens* should be incubated at 25°C.
		Pseudomonas species, Microbiologics 0162, and *P. putida*, Microbiologics 0627 and 0702, should be incubated at 30°C.
		P. aeruginosa, Microbiologics 0484, grows poorly on Nutrient Agar

Microorganism	Method	Notes
Ralstonia sp.	Method 1	
Raoultella sp.	Method 1	
Rhizopus sp.	Method 5	
Rhodococcus sp.	Method 2	
Rhodotorula sp.	Method 5	
Saccharomyces sp.	Method 5	Sabouraud Dextrose Emmons Agar is the best medium for growth of *Saccharomyces* sp.
Salmonella sp.	Method 1	
Scopulariopsis sp.	Method 5	
Serratia sp.	Method 1	
Shewanella sp.	Method 10	
Shigella sp.	Method 1	
Sphingobacterium sp.	Method 1	
Sphingomonas sp.	Method 1	Incubate at 25°C.
Sporidobolus sp.	Method 5	
Staphylococcus sp.	Method 1	The degree of resistance of *S. aureus*, Microbiologics 0158, to Vancomycin tends to decrease depending on age of culture, type of media, and number of subcultures. For best results, propagate strain on Brian Heart Infusion Agar with 4mcg/ml Vancomycin.
Stenotrophomonas sp.	Method 1	Incubate at 30°C.
Streptococcus sp.	Method 2	*S. criceti* must be incubated in a microaerophilic environment. *Streptococcus* sp., Microbiologics 0978, should be grown in CO_2.
		Streptococcus will also recover well on Columbia CNA Agar with 5% Sheep Blood.
Streptomyces sp.	Method 5	
Thermoanaerobacterium sp.	Method 4	Primary growth medium for *T. thermosaccharolyticum*, Microbiologics 0728, is Cooked Meat Medium. Incubation at 45°C for 72 hours is required. After initial growth, organism may be grown on Anaerobic Blood Agar which is incubated at 45°C for 72 hours in anaerobic atmosphere.
Trichoderma sp.	Method 5	
Trichophyton sp.	Method 5	Incubate for 7 to 14 days
Trichosporon sp.	Method 5	

Microorganism	Method	Notes
Ureaplasma sp.	Method 13	
Veillonella sp.	Method 4	
Vibrio sp.	Method 10	*V. alginolyticus*, Microbiologics 0819, does not recover well on Tryptic Soy Agar (Soybean Casein Digest Agar). For best results, grow on Marine Agar.
Virgibacillus sp.	Method 1	
Wallemia sebi	Method 5	
Yarrowia sp.	Method 5	
Yersinia sp.	Method 1	*Y. ruckeri*, Microbiologics 0785, should be incubated at 25°C.
Zygosaccharomyces sp.	Method 5	*Z. bailii*, Microbiologics 01011, does not grow well on nonselective Sheep Blood Agar, Nutrient Agar, or Tryptic Soy Agar (Soybean Casein Digest Agar).

3. The following information lists methods for growing microorganisms. When possible, more than one type of agar medium per method is listed.

Method 1

- Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar at 35°C in aerobic atmosphere for 24 to 48 hours.

Method 2

- Nonselective Sheep Blood Agar at 35°C in aerobic atmosphere for 24 to 72 hours. Growth of some species such as *Streptococcus* and *Arcanobacterium* are enhanced by CO_2 enrichment of the incubation atmosphere. 5% CO_2 is recommended for the culture of *Streptococcus pneumoniae* and other streptococcal species of the viridians group.

Method 3

- Chocolate Agar at 35°C in 5 to 7% CO_2 for 24 to 48 hours.

Method 4

- Anaerobic Blood Agar at 35°C in anaerobic environment for 48 to 72 hours.
- Some obligate anaerobes may require 5 to 7 days to demonstrate sufficient growth.
- Fresh prepared Nutrient Agar, Tryptic Soy Agar (Soybean Casein Digest Agar), and Standard Methods Agar (Plate Count Agar) are appropriate alternatives for some *Clostridium* species together with an additional period (24 hours) of incubation.

Method 5

- Sabouraud Dextrose Emmons Agar at 25°C in aerobic atmosphere for 2 to 7 days.
- Nonselective Sheep Blood Agar is an appropriate alternative.
- Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 6

- Chocolate Agar at 35°C in microaerophilic environment for 48 to 72 hours.

Method 7

- Lowenstein Jensen Agar or Middlebrook Agar at 35°C in 5 to 7% CO_2 or aerobic atmosphere. Incubation times may vary from 2 to 30 days. *M. fortuitum* subsp. *fortuitum*, *M. peregrinum* and *M. smegmatis* will also grow on Tryptic Soy Agar (Soybean Casein Digest Agar) as well as Lowenstein Jensen and Middlebrook Agar but additional incubation time may be required.

Method 8

- Buffered Charcoal Yeast Extract Agar at 35°C in aerobic atmosphere for 3 to 5 days.

Method 9

- V Agar or Chocolate Agar at 35°C in 5 to 7% CO_2 for 48 hours.

Method 10

- Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth (Soybean Casein Digest Agar), or 0.85% Saline. Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery.
- Grow on Tryptic Soy Agar (Soybean Casein Digest Agar) at 35°C in aerobic atmosphere for 24 to 48 hours. *Vibrio* sp. also grows on Marine Agar.

Method 11

- The primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in aerobic atmosphere for 48 hours. Transfer to either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood. Incubate at 35°C in 5 to 7% CO_2 for 48 hours. A few *Lactobacilli* species, such as *L. fermentum*, *L. paracasei* subsp. *paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. sakei* do not need to be started in Lactobacilli MRS broth. They may be plated directly to Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood and incubated at 35°C in 5 to 7% CO_2 for 48 hours.

Method 12

- Potato Dextrose Agar at 55°C in aerobic atmosphere for 24 to 48 hours.

Method 13

- Rehydrate 1 pellet of *Ureaplasma* sp. in SP4 Urea Broth. Alternatively, inoculate broth with a KWIK-STIK™. Make serial dilutions (for example, 1:10, 1:100, 1:1000, 1:10,000). Incubate at 35°C in aerobic atmosphere. As soon as the SP4 Urea Broth turns red (24 to 96 hours), sub 0.1 ml of broth to A-8 Agar and streak for isolation. Do not use cotton swabs or wooden sticks. Incubate A-8 agar at 35°C in anaerobic conditions for 4 to 6 days. In order to see colonies, examine plates microscopically.

Method 14

- Leeming Notman Agar at 30°C in aerobic atmosphere for 72 hours.

Method 15

- Chocolate agar, Sheep Blood Agar, Tryptic Soy Agar, and Bordet Gengou Agar with 15% Defibrinated Sheep Blood at 35°C in aerobic atmosphere for 24 to 48 hours. Standard Methods Agar (Plate Count Agar) or Nutrient Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 16

- Chocolate or Bordet Gengou Agar with 15% Defibrinated Sheep Blood at 35°C in aerobic atmosphere for 2 days to 1 week. *B. pertussis*, Microbiologics 0100, and *B. pertussis*, Microbiologics 0843, require Bordet Gengou Agar with 15% Defibrinated Sheep Blood.

Method 17

- Prepare and Use ISF (modified Infant Soy Formula) Broth using the following steps:
    1. Fill tubes with 10 ml Infant Soy Formula. Infant Soy Formula may be purchased at a grocery store.
    2. Place a four-penny nail in each tube. A four-penny nail is approximately 1.5 inches, or 38 mm, in length. It should contain steel or iron.
    3. Sterilize the broth.
    4. Inoculate ISF Broth with one LYFO DISK® or KWIK-STIK™.
    5. Grow at 55°C in anaerobic conditions for 48 hours. The broth will turn grey, indicating growth.
    6. Make two dilutions, 1:10 and 1:100.
    7. Sub with a swab to Sulfite Agar. Plate the undiluted sample and the 1:10 and 1:100 dilutions. It is necessary to plate the diluted samples because at higher concentrations the colonies are pin-point which makes colony characteristics difficult to see. Sulfite Agar is used for detecting thermophilic anaerobes which produce sulfite.
    8. Incubate the agar in anaerobic environment at 55°C for 48 hours to 7 days.

Method 18

- Inoculate Broth with LYFO DISK® or KWIK-STIK™. Prepare a 1:10 serial dilution using the broth. Incubate broth according to the table below. Then plate 0.2 ml of the broth culture to Agar. Incubate agar according to the table below. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Recommended Broth and Agar for Growth of Mycoplasma Species

Catalog Number	Microorganism	Broth	Broth Incubation Temperature / ATM / Time	Agar	Agar Incubation Temperature / ATM / Time
0156	*Mycoplasma hominis*	Mycoplasma	35°C O_2 48 hours	Mycoplasma	35°C 5 to 7% CO_2 4 to 6 days
0503	*Mycoplasma pneumoniae*	SP4 Glucose	35°C O_2 7 to 28 days	SP4 Glucose	35°C CO_2 (Candle Jar) 5 to 15 days
0504	*Mycoplasma orale*	Mycoplasma	35°C O_2 48 hours	Mycoplasma	35°C AN 3 to 6 days
01053	*Mycoplasma bovis*	Mycoplasma	35°C O_2 48 hours	Mycoplasma	35°C 5 to 7% CO_2 3 to 7 days
0151	*Ureaplasma parvum*	SP4 with Urea	35°C O_2 48 hours	A8	35°C AN 4 to 6 days

Method 19

- Sheep Blood Agar supplemented with Pyridoxal at 35°C in 5 to 7% CO_2 for 24 to 48 hours.