Recommended Culture Methods 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* species**	Method 36	
***Achromobacter* species**	Method 1	
***Acinetobacter* species**	Method 1	
***Actinobacillus* species**	Method 3	
***Actinomyces* species**	Method 4	
***Aerococcus* species**	Method 1	
***Aeromonas* species**	Method 2	Exceptions are *Aeromonas hydrophila*, Microbiologics 0870 and *Aeromonas salmoncida*
Aeromonas hydrophila, Microbiologics 0870	Method 31	
Aeromonas salmonicida	Method 32	
***Aggregatibacter* species**	Method 3	
***Alcaligenes* species**	Method 1	
***Alicyclobacillus* species**	Method 12	An exception is *Alicyclobacillus acidoterrestris*.
Alicyclobacillus acidoterrestris	Method 45	
***Alloiococcus* species**	Method 2	
***Alternaria* species**	Method 5	
***Amylomyces* species**	Method 5	
***Aneurinibacillus* species**	Method 1	
***Aquaspirillum* species**	Method 20	.
***Arcanobacterium* species**	Method 34	
***Arthrobacter* species**	Method 21	
***Aspergillus* species**	Method 5	An exception is *Aspergillus flavus*.
Aspergillus flavus	Method 46	
***Aureobasidum* species**	Method 5	
***Bacillus* species**	Method 49	
***Bacteroides* species**	Method 4	An exception is *Bacteroides ureolyticus*.
Bacteroides ureolyticus	Method 38	
***Bifidobacterium* species**	Method 4	An exception is *Bifidobacterium animalis* subsp. *animalis.*

Microorganism	Method	Notes
***Bifidobacterium animalis* subsp. *animalis*.**	Method 39	
Bordetella bronchiseptica	Method 15	
Bordetella parapertussis	Method 16	
Bordetella pertussis	Method 16	Exceptions are *Bordetella pertussis*, Microbiologics catalog numbers 0100 and 0843.
***Bordetella pertussis* Microbiologics 0100 and 0843**	Method 57	
***Brevibacillus* species**	Method 1	
***Brevundimonas* species**	Method 1	
***Brochothrix* species**	Method 21	
***Budvicia* species**	Method 21	
***Burkholderia* species**	Method 1	
***Campylobacter* species**	Method 6	
***Candida* species**	Method 5	
***Capnocytophaga* species**	Method 3	
***Cedecea* species**	Method 1	
***Cellulosimicrobium* species**	Method 1	
***Chaetomium* species**	Method 5	
***Chryseobacterium* species**	Method 1	An exception is *Chryseobacterium shigense*.
Chryseobacterium shigense	Method 22	
***Citrobacter* species**	Method 1	
***Cladosporium* species**	Method 5	
***Clostridium* species**	Method 40	Exceptions are *Clostridium difficile*, *Clostridium perfringens*, *Clostridium sordellii*, and *Clostridium tetani.*
Clostridium difficile	Method 4	
Clostridium perfringens	Method 41	An exception is *Clostridium perfringens*, Microbiologics 0318.
***Clostridium perfringens* Microbiologics 0318**	Method 63	
Clostridium sordellii	Method 4	
Clostridium tetani	Method 4	
***Corynebacterium* species**	Method 1	An exception is *Corynebacterium urealyticum*.
Corynebacterium urealyticum	Method 2	
***Cronobacter* species**	Method 1	
***Cryptococcus* species**	Method 62	An exception is *Cryptococcus gattii*.
Cryptococcus gattii	Method 47	
***Curtobacterium* species**	Method 1	
***Deinococcus* species**	Method 1	An exception is *Deinococcus radiophilus*, Microbiologics 01184.
***Deinococcus radiophilus* Microbiologics 01184**	Method 23	
***Delftia* species**	Method 1	

Microorganism	Method	Notes
***Desulfotomaculum* species**	Method 17	
***Edwardsiella* species**	Method 1	
***Eggerthella* species**	Method 4	
***Eikenella* species**	Method 3	
***Elizabethkingia* species**	Method 1	
***Enterobacter* species**	Method 1	
***Enterococcus* species**	Method 1	
***Erysipelothrix* species**	Method 2	
Escherichia coli	Method 1	
Eurotium rubrum	Method 5	
***Exiguobacterium* species**	Method 1	
***Finegoldia* species**	Method 42	
***Fluoribacter* species**	Method 8	
***Fusarium* species**	Method 5	
***Fusobacterium* species**	Method 4	An exception is *Fusobacterium mortiferum*, Microbiologics 01191.
***Fusobacterium mortiferum* Microbiologics 01191**	Method 39	
***Gardnerella* species**	Method 9	
***Gemella* species**	Method 4	
***Geobacillus* species**	Method 24	An exception is *Geobacillus stearothermophilus*, Microbiologics 0137.
Geobacillus stearothermophilus	Method 64	
***Geotrichum* species**	Method 5	
Granulicatella adiacens	Method 19	
***Haemophilus* species**	Method 3	
***Hafnia* species**	Method 1	
***Hanseniaspora* species**	Method 5	
Herminiimonas species	Method 20	
***Issatchenkia* species**	Method 5	
***Kingella* species**	Method 33	
***Klebsiella* species**	Method 1	
***Kloeckera* species**	Method 5	
***Kocuria* species**	Method 1	An exception is *Kocuria rosea*.
Kocuria rosea	Method 21	
***Lactobacillus* species**	Method 65	Exceptions are *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus gasseri*, and *Lactobacillus leichmanni*.
Lactobacillus acidophilus	Method 11	
Lactobacillus casei	Method 11	
Lactobacillus gasseri	Method 11	
Lactobacillus leichmannii	Method 11	

Microorganism	Method	Notes
***Lactococcus* species**	Method 2	
***Leclercia* species**	Method 1	
***Legionella* species**	Method 8	
***Listeria* species**	Method 1	
***Lysinibacillus* species**	Method 1	
***Macrococcus* species**	Method 1	
Magnusiomyces capitatus	Method 5	
***Malassezia* species**	Method 14	
***Mannheimia* species**	Method 1	
***Methylobacterium* species**	Method 25	An exception is *Methylobacterium extorquens*.
Methylobacterium extorquens	Method 26	
Meyerozyma guilliermondii	Method 5	
***Microbacterium* species**	Method 22	
***Micrococcus* species**	Method 1	An exception is *Micrococcus luteus*, Microbiologics 0337 and *Micrococcus luteus*, Microbiologics 0689.
***Micrococcus luteus*, Microbiologics 0337**	Method 27	
***Micrococcus luteus*, Microbiologics 0689**	Method 67	
Microsporum species	Method 5	Exceptions are *Microsporum canis* and *Microsporum gypseum*.
Microsporum canis	Method 48	
Microsporum gypseum	Method 73	
***Moraxella* species**	Method 2	
***Morganella* species**	Method 1	
Mucor racemosus	Method 5	
***Mycobacterium* species**	Method 13	Exceptions are *Mycobacterium fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium haemophilum*, and *Mycobacterium smegmatis*.
Mycobacterium fortuitum	Method 7	
Mycobacterium haemophilum	Method 18	
Mycobacterium peregrinum	Method 7	
Mycobacterium smegmatis	Method 7	
Mycoplasma bovis	Method 60	
Mycoplasma hominis	Method 58	
Mycoplasma pneumoniae	Method 59	
***Myroides* species**	Method 2	
***Neisseria* species**	Method 37	
***Nocardia* species**	Method 81	
***Novosphingobium* species**	Method 21	
***Ochrobactrum* species**	Method 1	
***Oligella* species**	Method 2	

Microorganism	Method	Notes
***Paecilomyces* species**	Method 5	
***Paenibacillus* species**	Method 1	An exception is *Paenibacillus larvae*.
Paenibacillus larvae	Method 22	
***Parabacteroides* species**	Method 4	
***Parvimonas* species**	Method 43	
***Pasteurella* species**	Method 2	
***Pediococcus* species**	Method 11	An exception is *Pediococcus damnosus*.
Pediococcus damnosus	Method 55	
***Penicillium* species**	Method 5	
***Peptoniphilus* species**	Method 42	
***Peptostreptococcus* species**	Method 4	
***Plesiomonas* species**	Method 1	
Pluralibacter gergoviae	Method 1	
***Porphyromonas* species**	Method 43	
***Prevotella* species**	Method 43	
***Propionibacterium* species**	Method 44	
***Proteus* species**	Method 1	An exception is *Proteus hauseri*.
Proteus hauseri	Method 27	
***Prototheca* species**	Method 5	
***Providencia* species**	Method 1	
***Pseudomonas* species**	Method 1	Exceptions are *Pseudomonas aeruginosa*, Microbiologics 0484, *Pseudomonas benneri, Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas protegens, Pseudomonas mosselii*, *Pseudomonas putida*, and *Pseudomonas* species, Microbiologics 0162.
***Pseudomonas aeruginosa*, Microbiologics 0484**	Method 28	
Pseudomonas brenneri	Method 21	
Pseudomonas fluorescens	Method 21	
Pseudomonas fragi	Method 21	
Pseudomonas mosselii	Method 21	
Pseudomonas protegens	Method 21	
***Pseudomonas putida*, Microbiologics 0627 and 0702**	Method 22	
***Pseudomonas* species, Microbiologics 0162**	Method 22	
***Ralstonia* species**	Method 1	An exception is *Ralstonia pickettii*, Microbiologics 01197.
***Ralstonia pickettii*, Microbiologics 01197**	Method 29	
***Raoultella* species**	Method 1	
***Rhizopus* species**	Method 5	
Rhizobium radiobacter	Method 21	

Microorganism	Method	Notes
***Rhodococcus* species**	Method 2	
***Rhodotorula* species**	Method 5	
***Saccharomyces* species**	Method 50	
***Salmonella* species**	Method 1	
***Scopulariopsis* species**	Method 5	
***Serratia* species**	Method 1	
***Shewanella* species**	Method 1	Exceptions are *Shewanella haliotis* and *Shewanella putrefaciens*.
Shewanella haliotis	Method 74	
Shewanella putrefaciens	Method 75	
***Shigella* species**	Method 1	
***Sphingobacterium* species**	Method 1	
***Sphingomonas* species**	Method 21	
***Sporidobolus* species**	Method 5	
***Staphylococcus* species**	Method 1	An exception is *Staphylococcus aureus*, Microbiologics 0158.
***Staphylococcus aureus*, Microbiologics 0158**	Method 30	
***Stenotrophomonas* species**	Method 22	
***Streptococcus* species**	Method 34	Exceptions are *Streptococcus criceti*, *Streptococcus* species, Microbiologics 0978, and *Streptococcus pneumoniae*.
Streptococcus criceti	Method 35	
Streptococcus pneumoniae	Method 66	
***Streptococcus* species Microbiologics 0978**	Method 66	
Streptomyces species	Method 5	
Talaromyces pinophilus	Method 5	
***Thermoanaerobacterium* species**	Method 56	
***Trichoderma* species**	Method 5	
***Trichophyton* species**	Method 51	
***Trichosporon* species**	Method 5	
Trueperella pyogenes	Method 34	
***Ureaplasma* species**	Method 61	
***Veillonella* species**	Method 4	
***Vibrio* species**	Method 10	An exception is *Vibrio alginolyticus.*
***Vibrio alginolyticus*, Microbiologics 0819**	Method 54	
***Virgibacillus* species**	Method 1	
Wallemia mellicola	Method 5	
***Yarrowia* species**	Method 5	
***Yersinia* species**	Method 1	An exception is *Yersinia ruckeri*, Microbiologics 0785.

Microorganism	Method	Notes
***Yersinia ruckeri*, Microbiologics 0785**	Method 21	
***Zygosaccharomyces* species**	Method 5	Exceptions are *Zygosaccharomyces rouxii*, Microbiologics 0803; *Zygosaccharomyces bisporus*, Microbiologics 0960; and *Zygosaccharomyces parabailii*, Microbiologics 01011.
***Zygosaccharomyces bisporus*, Microbiologics 0960**	Method 53	
***Zygosaccharomyces rouxii*, Microbiologics 0803**	Method 53	
***Zygosaccharomyces parabailii*, Microbiologics 01011**	Method 52	

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

Method 1

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 2

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 3

Media	Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	24 to 48 hours

Method 4

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: Some obligate anaerobes may require 5-7 days to demonstrate sufficient growth.

Method 5

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: 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

Media	Chocolate Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	48 to 72 hours

Note: Do not open the inoculated agar medium petri plate for the first 48 hours.

Method 7

Media	Lowenstein Jensen Agar or Middlebrook Agar
Temperature	35°C
Atmosphere	5 to 7% CO_2 or aerobic atmosphere.
Growth Time	2 to 30 days

Note: *M. fortuitum* subssp. *fortuitum*, *M. smegmatis*, *M. peregrinum* 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

Media	Buffered Charcoal Yeast Extract Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	3 to 5 days

Method 9

Media	V Agar or Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	48 hours

Method 10

Media	Tryptic Soy Agar (Soybean Casein Digest Agar) or Marine Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 35°C for 24 to 48 hours.
Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery when grown on the recommended media.

Method 11

Media	Phase 1: MRS (Man, Rogosa, Sharpe) Broth Phase 2: Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 hours Phase 2: 48 hours

Note: For Phase 1, the primary growth medium is MRS (Man, Rogosa, Sharpe) Broth. Incubate at 35°C in aerobic atmosphere for 48 hours. For Phase 2, transfer to either Columbia CNA with Sheep Blood or Tryptic Soy Agar with Sheep Blood using a sterile swab or pipette. Incubate at 35°C in 5 to 7% carbon dioxide for 48 hours.

Method 12

Media	Potato Dextrose Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 13

Media	Lowenstein Jensen Agar or Middlebrook Agar
Temperature	35°C
Atmosphere	5 to 7% CO_2 or Aerobic
Growth Time	May require up to one month of incubation.

Method 14

Media	Leeming Notman Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	72 hours

Method 15

Media	Chocolate agar, Sheep Blood Agar, Tryptic Soy Agar, and Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Standard Methods Agar (Plate Count Agar) or Nutrient Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 16

Media	Chocolate agar or Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	2 days to 1 week

Method 17

Media	Phase 1: ISF (modified Infant Soy Formula) Broth Phase 2: Sulfite Agar
Temperature	Phase 1: 55°C Phase 2: 55°C
Atmosphere	Phase 1: Anaerobic Phase 2: Anaerobic
Growth Time	Phase 1: 48 hours Phase 2: 48 hours to 7 days

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. Subculture 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

Media	Middlebrook 7H11 Agar
Temperature	30°C
Atmosphere	5 to 7% CO_2
Growth Time	3 to 4 weeks

Note: An X factor strip must be placed on the agar in order for the organism to grow.

Method 19

Media	Sheep Blood Agar supplemented with Pyridoxal
Temperature	35°C
Atmosphere	5 to 7% CO_2
Growth Time	24 to 48 hours

Method 20

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	6 days

Method 21

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 22

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 23

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 24

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 25

Media	Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 days

Method 26

Media	Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 days

Note: Alternatively, *M. extorquens* may be grown on R2A Agar in 72 hours at 30°C.

Method 27

Media	Tryptic Soy Agar (Soybean Casein Digest Agar) or Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 28

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, or Standard Methods Agar (Plate Count Agar)
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 29

Media	Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar), or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 30

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: 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 Brain Heart Infusion Agar with 4mcg/ml Vancomycin.

Method 31

Media	Nonselective Sheep Blood Agar
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 32

Media	Nonselective Sheep Blood Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Method 33

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	5 to 10% CO_2
Growth Time	24 to 72 hours

Method 34

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 72 hours

Note: *Streptococcus* will also recover well on Columbia CNA Agar with 5% Sheep Blood.
Note: Growth of some species such as *Streptococcus*, *Arcanobacterium* and *Trueperella* is enhanced by enrichment of the incubation atmosphere with carbon dioxide. 5% carbon dioxide is recommended for the culture of *Streptococcus pneumoniae* and other streptococcal species of the viridans group.

Method 35

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	24 to 72 hours

Method 36

Media	Chocolate Agar
Temperature	25°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	3 to 4 days

Method 37

Media	Chocolate Agar
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	24 to 48 hours

Note: Do not open the inoculated agar medium petri plate for the first 48 hours if using a candle jar.

Method 38

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	2 to 5 days

Note: *B. ureolyticus* colonies are very small. Several subculture plates may need to be inoculated to have sufficient quantity of the microorganism for testing.

Method 39

Media	Anaerobic Blood Agar, Tryptic Soy Agar (Soybean Casein Digest Agar)
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Method 40

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: 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. *Clostridium* species may have reduced recovery when using the alternative agars.

Method 41

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	24 hours

Note**:** Tryptic Soy Agar (Soybean Casein Digest Agar) and Standard Methods Agar are appropriate alternatives together with an additional period (24 hours) of incubation. Using alternative agars may result in reduced recovery for *Clostridium* species.

Method 42

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	3 to 4 days

Method 43

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	5 to 7 days

Method 44

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	3 to 5 days

Method 45

Media	Potato Dextrose Agar
Temperature	45°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 46

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.
Note: Nutrient Agar, Tryptic Soy Agar, and Potato Dextrose Agar are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 47

Media	Sabouraud Dextrose Emmons Agar or Malt Extract Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Method 48

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.
Note: Nutrient Agar, Tryptic Soy Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 49

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Some Bacillus spp. demonstrate better recovery on subculture when the stock organism growth is maintained at room temperature rather than 2° to 8°C.

Method 50

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Method 51

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7 to 14 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.
Note: 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 52

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Potato Dextrose Agar and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 53

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Nonselective Sheep Blood Agar is an appropriate alternative.
Note: Nutrient Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives together with an additional period (24 hours) of incubation.

Method 54

Media	See notes below for rehydration instructions; Marine Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 35°C for 24 to 48 hours.
Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery.

Method 55

Media	See note below for important directions. Phase 1: MRS (Man, Rogosa, Sharpe) Broth Phase 2: MRS Agar
Temperature	Phase 1: 25°C Phase 2: 25°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% Carbon Dioxide
Growth Time	Phase 1: 48 to 72 hours Phase 2: 72 to 96 hours.

Note: For Phase 1, *P. damnosus* may be grown in MRS broth at 25ºC for 48 to 72 hours. When the broth becomes cloudy, begin Phase 2 by subculturing the broth to MRS agar using a sterile swab or pipette. Incubate the MRS agar at 25ºC in 5 to 7% carbon dioxide for 72 to 96 hours.
Note: Alternatively, the lyophilized microorganism may be grown directly on MRS Agar at 25ºC in 5 to 7% carbon dioxide for 5 to 7 days.

Method 56

Media	See note below for important directions. Phase 1: Cooked Meat Medium Phase 2: Anaerobic Blood Agar
Temperature	Phase 1: 45°C Phase 2: 45°C
Atmosphere	Phase 1: Aerobic Phase 2: Anaerobic
Growth Time	Phase 1: 72 hours Phase 2: 3 to 5 days

Note: Primary growth medium for *T. thermosaccharolyticum*, Microbiologics 0728, is Cooked Meat Medium. During Phase 1, incubation at 45°C for 72 hours is required. During Phase 2, the organism is transferred to Anaerobic Blood Agar which is incubated anaerobically at 45°C for 3 to 5 days.

Method 57

Media	Bordet Gengou Agar with 15% Defibrinated Sheep Blood
Temperature	35°C
Atmosphere	Aerobic
Growth Time	2 days to 1 week

Method 58

Media	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% CO_2
Growth Time	Phase 1: 48 hours Phase 2: 4 to 6 days

Note: Inoculate Broth with LYFO DISK® or KWIK-STIK™. For Phase 1, prepare a 1:10 serial dilution using Mycoplasma Broth. Incubate the broth aerobically at 35°C for 48 hours. After incubation, begin Phase 2 by plating 0.2 ml of the broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% CO_2 at 35°C for 3 to 7 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 59

Media	See note below for important directions. Phase 1: SP4 Glucose Broth Phase 2: SP4 Glucose Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: CO_2 (Candle Jar)
Growth Time	Phase 1: 7 to 28 days Phase 2: 5 to 15 days

Note: Inoculate Broth with LYFO DISK® or KWIK-STIK™. For Phase 1, prepare a 1:10 serial dilution using SP4 Glucose Broth. Incubate broth aerobically at 35°C for 7 to 28 days until the broth turns yellow. Then plate 0.2 ml of the broth culture to SP4 Glucose Agar. Incubate agar in a candle jar for 5 to 15 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 60

Media	See note below for important directions. Phase 1: Mycoplasma Broth Phase 2: Mycoplasma Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: 5 to 7% CO_2
Growth Time	Phase 1: 48 hours Phase 2: 3 to 7 days

Note: Inoculate Broth with LYFO DISK® or KWIK-STIK™. For Phase 1, prepare a 1:10 serial dilution using Mycoplasma Broth. Incubate the broth aerobically at 35°C for 48 hours. After incubation begin

Phase 2 by plating 0.2 ml of the broth culture to Mycoplasma Agar. Incubate agar in 5 to 7% CO_2 at 35°C for 3 to 7 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 61

Media	See note below for important directions. Phase 1: SP4 Urea Broth Phase 2: A8 Agar
Temperature	Phase 1: 35°C Phase 2: 35°C
Atmosphere	Phase 1: Aerobic Phase 2: Anaerobic
Growth Time	Phase 1: 48 hours Phase 2: 4 to 6 days

Note: Inoculate Broth with LYFO DISK® or KWIK-STIK™. For Phase 1, prepare a 1:10 serial dilution using SP4 Urea Broth. Incubate the broth aerobically at 35°C for 24 to 96 hours. As soon as the SP4 Urea Broth turns red, begin Phase 2 by plating 0.1 ml of the broth to A8 Agar and streak for isolation. Incubate A8 agar anaerobically at 35°C for 4 to 6 days. Do not use cotton swabs or wooden sticks. In order to see colonies, examine plates microscopically.

Method 62

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 7 days

Note: Cryptococcus species grows poorly on Nonselective Sheep Blood Agar.
Note: 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 63

Media	Anaerobic Blood Agar
Temperature	35°C
Atmosphere	Anaerobic
Growth Time	48 to 72 hours

Note: Tryptic Soy Agar (Soybean Casein Digest Agar) is an appropriate alternative together with an additional period (24 hours) of incubation time. Using the alternative agar may result in reduced recovery for *Clostridium* species.

Method 64

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	55°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 65

Media	Nonselective Sheep Blood Agar or Columbia CNA with 5% Sheep Blood
Temperature	35°C
Atmosphere	5 to 7% Carbon Dioxide
Growth Time	48 hours

Note: Alternatively, the strain may be grown in MRS (Man, Rogosa, Sharpe) Broth in an aerobic atmosphere for 48 hours. Transfer to either Columbia CNA with Sheep Blood or Nonselective Sheep Blood Agar. Incubate at 35°C in 5 to 7% carbon dioxide for 48 hours.

Method 66

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	5% Carbon Dioxide
Growth Time	24 to 72 hours

Note: *Streptococcus* will also recover well on Columbia CNA with 5% Sheep Blood.

Method 67

Media	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Alternatively, the microorganism may be grown on Standard Methods Agar (Plate Count Agar) for a minimum of 72 hours.

Method 68

Media	Chocolate Agar
Temperature	35°C
Atmosphere	Microaerophilic
Growth Time	48 hours

Method 69

Media	Malt Extract Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	5 to 7 days

Method 70

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Method 71

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	7-14 days

Method 72

Media	Nonselective Sheep Blood Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	24 hours

Method 73

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 4 weeks

Note: Nonselective Sheep Blood Agar,
Malt Agar, Nutrient Agar, Tryptic Soy Agar, Potato Dextrose Agar, and Standard Methods Agar (Plate Count Agar) are appropriate alternatives.

Method 74

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar or Standard Methods Agar (Plate Count Agar)
Temperature	30°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 30°C for 24 to 48 hours.
Note: Rehydration with water may result in decreased or no recovery. Rehydration with fluid provided in the KWIK-STIK™ unit provides satisfactory recovery when transferred to the recommended media.

Method 75

Media	Tryptic Soy Agar (Soybean Casein Agar) or Nonselective Sheep Blood Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	24 to 48 hours

Note: Rehydrate in sterile Brain Heart Infusion Broth, Tryptic Soy Broth, or 0.85% Saline. Transfer a portion of hydrated material immediately to agar. Incubate aerobically at 25°C for 24 to 48 hours.
Note: Rehydration with water or the fluid provided in the KWIK-STIK™ unit may result in decreased or no recovery.

Method 76

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	One week

Method 77

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	2 to 4 days

Method 78

Media	Sabouraud Dextrose Emmons Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	48 to 72 hours

Method 79

Media	Potato Dextrose Agar
Temperature	25°C
Atmosphere	Aerobic
Growth Time	3 to 7 days

Method 80

Media	Nutrient
Temperature	25°C
Atmosphere	Aerobic
Growth Time	3 to 5 days

Method 81

Media	Tryptic Soy Agar (Soybean Casein Digest Agar), Nonselective Sheep Blood Agar, Standard Methods Agar (Plate Count Agar) or Nutrient Agar
Temperature	35°C
Atmosphere	Aerobic
Growth Time	4 to 5 days

Note: Very small colonies develop within 48 hours. The colony morphology does not fully develop until 4 to 5 days.